(54) Title: BICYCLIC CB2 CANNABINOID RECEPTOR LIGANDS

(57) Abstract: The present invention relates to non-classical cannabinoids that are ligands of the peripheral cannabinoid receptor CB2, and to pharmaceutical compositions thereof comprising as an active ingredient novel (+) α-pinene derivatives, which are useful for prevention and treatment of autoimmune diseases including but not limited to rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, myasthenia gravis, diabetes mellitus type I, psoriasis, tissue rejection in organ transplants, malabsorption syndromes such as celiac disease, pulmonary diseases such as asthma and Sjögren's syndrome, inflammation including inflammatory bowel disease, pain including peripheral, neuropathic and referred pain, muscle spasticity, cardiovascular disorders including arrhythmia, hypertension and myocardial ischemia, neurological disorders including stroke, migraine and cluster headaches, neurodegenerative diseases including Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, Huntington's chorea, prion-associated neurodegeneration, CNS poisoning and certain types of cancer.

BICYCLIC CB2 CANNABINOID RECEPTOR LIGANDS

FIELD OF THE INVENTION

The present invention relates to (+) α-pinene derivatives that are ligands of the peripheral cannabinoid receptor CB2, and to pharmaceutical compositions thereof, which are useful for prevention and treatment of autoimmune diseases and related disorders, inflammation, pain, muscle spasticity, cardiovascular disorders, neurological disorders, neurodegenerative diseases, CNS poisoning and certain types of cancer.

BACKGROUND OF THE INVENTION

Cannabis sativa preparations have long been known as therapeutic agents to treat various diseases (Mechoulam, R. in "Cannabinoids as Therapeutic Agents" CRC Press, Boca Raton, Fla., 1-19, 1986). The native active constituent, Delta 9-tetrahydrocannabinol (Δ^9-THC), is prescribed today, under the generic name Dronabinol, as an anti-emetic and for enhancement of appetite, mainly in AIDS patients. However, separation between the clinically undesirable psychotropic effects and the therapeutically desirable effects, such as vascular hypotension and immunomodulation, has only recently been accomplished. The discovery of two cannabinoid receptors, CB1 and CB2, has helped to elucidate the diverse cannabinoid effects.

The receptors were shown to have seven transmembrane structures G-protein coupled that share 44% amino acid sequence homology but differ in tissue specificity (Munro, S., Thomas, K.L. & Abu-Shaar, M., Nature 365: 61-5, 1993). Both receptors exert their effect by negative regulation of adenylyl cyclase activity through the pertussis toxin-sensitive GTP-binding protein. They were also shown to activate the mitogen activated protein kinase (MAPK) in certain cell types (Parolaro, D., Life Sci. 65: 637-44, 1999).

The CB1 receptor is expressed mainly in the CNS and to a lesser extent in other tissues. The CB2 receptor is expressed mostly in peripheral tissue associated with immune functions, including macrophages, B and T cells, as well as in peripheral nerve terminals and

on mast cells (Pertwee, R.G., Prog. Neurobiol. 63: 569-611, 2001). While the effects mediated by CB1, primarily in the CNS, have been thoroughly investigated those mediated by CB2 are only now being elucidated.

5   The neuroanatomical distribution of the receptors was determined using radiolabeled THC analogs such as [^{3}H]CP-55940 (Elphick, M.R. & Egertova, M., Phil. Trans. R. Soc. Lond. B Biol. Sci. 356: 381-408, 2001). Highest concentrations of cannabinoid binding site, specifically CB1 receptor, are found in the basal ganglia and cerebellum, regions of the brain that are involved in movement. A subpopulation of the receptors is expressed in the peripheral terminals of the dorsal root ganglion, hence the analgesic effects of cannabinoids. Additional

10   techniques, including immunohistochemistry, *in situ* hybridization assays using specific transcripts (Galiegue, S. et al., Eur. J. Biochem. 232: 54-61, 1995) and knockout mice (Buckley, N.E. et al., Eur. J. Pharmacol. 396: 141-9, 2000) have been used to contribute to the understanding of the receptors' expression patterns and function. The CB2 receptor is not expressed in the brain but is particularly abundant in immune tissues, with an expression level

15   10-100 fold higher than that of CB1. In spleen and tonsils, the CB2 mRNA content was equivalent to that of CB1 mRNA in the central nervous system. Among the main human blood cell subpopulations, the distribution pattern of the CB2 mRNA displayed important variations with higher levels in B-cells than in natural killer cells or monocytes and low levels in polymorphonuclear neutrophil cells, T8 cells and T4 cells.

20   CB1 knockout mice have been shown to be unresponsive to cannabinoids in behavioral assays providing molecular evidence that the psychotropic effects, including sedation, hallucinations and delirium and anti-nociception are manifested through activation of the CB1 receptor, present primarily in the CNS. Analysis of the CB2 knockout mouse has corroborated the evidence for the function of CB2 receptors in modulating the immune

25   system. CB2 does not affect immune cell development and differentiation as determined by FACS analysis of cells from the spleen, lymph node and thymus from CB2 knockout mice, but rather mediates the suppressive effect of Δ^9-THC.

Due to the restricted expression of the CB2 receptor in subsets of immune cells and neurons, selective CB2 ligands have therapeutic value (Pertwee, R.G., Curr. Med. Chem. 6:

30   635-64, 1999). Of particular interest are those compounds with high affinity and high

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specificity for the CB2 receptor. These compounds could afford the benefits of CB2 agonism while avoiding the adverse side effects seen in compounds with affinity for the CB1 receptor. Such compounds could be effective in the treatment of autoimmune diseases including but not limited to multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, myasthenia

5   gravis, diabetes mellitus type I, inflammatory bowel disease or irritable bowel syndrome, psoriasis and other immune related disorders including but not limited to tissue rejection in organ transplants, malabsorption syndromes such as celiac disease, pulmonary diseases such as asthma and Sjögren's syndrome.

The discovery of cannabinoid receptors and the more recent identification of

10   endocannabinoids, endogenous ligands capable of activating the CB receptors, has led to the understanding of the multiplicity of effects exerted by cannabinoids and related compounds. On top of a general neuroprotective effect of certain cannabinoid agonists more specific applications can be found. Thus, for example, evidence for the tonic control of spasticity by the endocannabinoid system suggests that cannabinoid agonists may help in the treatment of

15   muscle spasm and tremor in multiple sclerosis (Baker D. et al., FASEB J. 15: 300-2, 2001), in addition to the possible moderation of the disease by immunomodulation through an action on CB2 receptors expressed by immune cells. Cannabinoid agonists may also prove to be of help in the treatment muscle spasm in cancer and HIV/AIDS (Hall W.D., Degenhardt L.J. & Currow D., Med. J. Aust. 175: 39-40, 2001) and of neuromuscular disorders.

20   Activation of the CB1 receptor has therapeutic benefits in the treatment of pain and inflammation in addition to the sedative and undesirable psychotropic effects. Compounds that selectively activate the CB2 receptor have potential as immunomodulatory agents and may offer a therapeutic approach to treating autoimmune diseases and related disorders. In addition, selective CB2 receptor agonists have been shown to be useful in the treatment of

25   inflammation and pain, myocardial ischemia and certain types of cancer. THC, as well as the two major endogenous ligands identified so far, arachidonoylethanolamide (anandamide or AEA) (Devane, W.A. et al., Science 258: 1946-9, 1992) and 2-arachidonylglycerol (2-AG) (Sugiura, T. et al., Biochem. Biophys. Res. Commun. 215: 89-97, 1995) exert most of their effects by binding to both cannabinoid receptors.

30   Several synthetic compounds have been shown to bind to the CB2 receptor with a higher affinity than to the CB1 receptor (Pertwee, R.G., Expert Opin. Investig. Drugs 9: 1553-71, 2000). Cannabinoid receptor agonists comprise four main groups of compounds. The

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classic cannabinoids maintain the dibenzopyran ring system of THC while the non-classical cannabinoids include bicyclic or tricyclic analogs lacking the pyran ring. The aminoalkylindoles and analogs make up the third family and the endocannabinoids including anandamide and other fatty acid derivatives comprise the fourth family. For instance, L-759656 is a classical cannabinoid analog and HU-308 is a bicyclic analog. Both have CB2/CB1 binding affinity ratios of 300-400 and both have been shown to behave as potent and specific CB2 agonists in functional assays (Hanuš, L. et al., Proc. Natl. Acad. Sci. USA 96: 14228-33, 1999; Ross, R.A. et al., Br. J. Pharmacol. 126: 665-72, 1999).

The evidence linking CB2 receptor activation with therapeutic properties is manifold. The involvement of cannabinoids in cardioprotection, against ischemic and reperfusion effects including arrhythmia specifically through activation of the CB2 has recently been described in PCT patent application WO 01/28588 and by Krylatov et al. (Krylatov A.V. et al., Bull. Exp. Biol. Med. 131: 523-5, 2001), the disclosures of which are hereby incorporated by reference. Certain tumors, especially gliomas, express CB2 receptors. Guzman et al. (Galve-Roperh, I. et al., Nat. Med. 6: 313-9, 2000; Guzman, M., Sanchez, C., Galve-Roperh, I., J. Mol. Med. 78: 613-25, 2001) have shown that THC and WIN55212-2, the former a natural ligand and the latter a synthetic cannabinoid, induce the regression or eradication of malignant brain tumors in animals. The rat glioma C6 cell line expresses CB2 and on the basis of studies with selective CB antagonists, it has been proposed that activation of either of the receptors may trigger apoptosis.

The role of the endocannabinoid system in immunosuppression is the focus of many studies (Berdyshev, E.V., Chem. Phys. Lipids 108: 169-90, 2000). Anandamide (AEA), Palmitoylethanolamide (PEA) and 2-AG were shown to down-regulate the immune response in a variety of experimental systems and function as anti-inflammatory and immunosuppressive agents.

THC is known for its analgesic properties. The two major endogenous ligands, AEA and 2-AG have also been shown to act as analgesic agents and can exert their effects by binding to both cannabinoid receptors (Calignano, A. et al., Nature 394: 277-81, 1998). Therefore agonists of the CB2 receptor or putative CB2-like receptors are useful as agents for suppressing peripheral, neuropathic and referred pain. Moreover, a CB2 receptor ligand may be protective against CNS poisoning.

US Patent 5,434,295 discloses a family of novel 4-phenyl pinene derivatives, and teaches how to utilize said compounds in pharmaceutical compositions useful in treating various pathological conditions associated with damage to the central nervous system. This disclosure neither teaches nor suggests that any of those are selective for peripheral

5   cannabinoid receptors. International patent application WO 01/32169 (and Hanuš, L. et al., ibid) discloses the bicyclic compound HU-308 as a CB2 specific agonist and exemplifies its use in the treatment of pain and inflammation, autoimmune diseases, gastrointestinal disorders and as a hypotensive agent.

US Patent 6,013,648 discloses indole derivatives that are CB2 specific agonists and

10   may be used for preparing immunomodulating drugs. International patent application WO 01/28497 discloses novel bicyclic cannabinoid analogs that exhibit high affinity for the CB2 receptor. It is apparent to the skilled artisan that the compounds in said patent are of a stereochemical orientation wherein C-1, C-4 and C-5 are R, when referring to the nomenclature adopted in the present disclosure. However the corresponding (+) α-pinene

15   derivatives have not been synthesized and their therapeutic activity is unknown.

It is understood that the present invention explicitly excludes known compounds, including those disclosed in US Patent Nos. 4,282,248 and 5,434,295 and in international patent application WO 01/32169, though certain novel properties of these compounds are claimed as such.

20   **SUMMARY OF THE INVENTION**

It is an object of the present invention to provide compounds with specific binding affinity toward the peripheral cannabinoid receptor CB2, thereby providing new therapeutic entities comprising specific CB2 binding ligands. It is another object of the present invention to provide CB2 binding ligands, capable of exerting their CB2 receptor-specific effects *in*

25   *vivo*.

According to a first embodiment of the present invention, we disclose CB2 binding compounds having the general formula (I):

Formula I
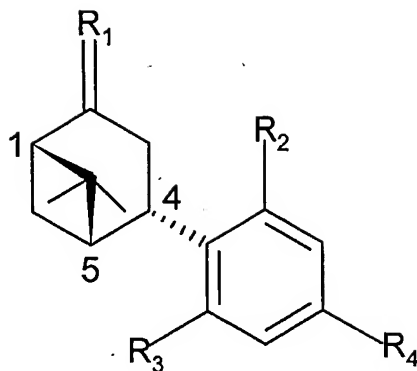
having a specific stereochemistry wherein C-5 is S, the protons at C-1 and C-5 are cis in
relation to one another and the protons at C-4 and C-5 are trans; and wherein:

5   R_1 is selected from the group consisting of

(a) O,

(b) S,

(c) $C(R')_2$ wherein each R' is independently selected from the group consisting of
hydrogen, cyano, -OR", $-N(R")_2$, a saturated or unsaturated, linear, branched or cyclic
10   C_1-C_6 alkyl, C_1-C_6 alkyl-OR" or C_1-C_6 alkyl-$N(R")_2$ wherein at each occurrence R" is
independently selected from the group consisting of hydrogen, C(O)R''', $C(O)N(R''')_2$,
C(S)R''', saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl, C_1-C_6 alkyl-
OR''', and C_1-C_6 alkyl-$N(R''')_2$, wherein at each occurrence R''' is independently
selected from the group consisting of hydrogen or saturated or unsaturated, linear,
15   branched or cyclic C_1-C_{12} alkyl, and

(d) NR^a wherein R^a is selected from the group consisting of a hydrogen, R" and -OR"
wherein R" is as previously defined;

R_2 and R_3 are each independently selected from the group consisting of

(a) halogen,

20   (b) -R", -OR", $-N(R")_2$, -SR", -S(O)(O)NR", wherein at each occurrence R" is as
previously defined, and

(c) $-S(O)R^b$, $-S(O)(O)R^b$, $-S(O)(O)OR^b$ wherein R^b is selected from the group
consisting of hydrogen, saturated or unsaturated, linear, branched or cyclic C_1-C_6

alkyl, C_1-C_6 alkyl-OR", and C_1-C_6 alkyl-N(R")$_2$, wherein R" is as previously defined; and

R_4 is selected from the group consisting of

(a) R wherein R is selected from the group consisting of hydrogen, OR''', OC(O)R''', C(O)OR''', C(O)R''', OC(O)OR''', N(R''')$_2$, NC(O)R''', NC(O)OR''', C(O)N(R''')$_2$, NC(O)N(R''')$_2$, SR''', and C(S)R''', wherein at each occurrence R''' is as previously defined,

(b) a saturated or unsaturated, linear, branched or cyclic C_1-C_{12} alkyl-R wherein R is as previously defined,

(c) an aromatic ring which can be further substituted at any position by a halogen, CN, NO_2 and R wherein R is as previously defined, and

(d) a saturated or unsaturated, linear, branched or cyclic C_1-C_{12} alkyl optionally terminated by an aromatic ring which can be further substituted as defined in (c).

According to a currently preferred embodiment, we now disclose a CB2 binding compound of the general formula I wherein R_1 is O, R_2 and R_3 are OH, and R_4 is 1,1-dimethylheptyl.

According to another currently preferred embodiment, we disclose a CB2 binding compound of the general formula I wherein R_1 is O, R_2 and R_3 are OCH_3, and R_4 is 1,1-dimethylheptyl.

According to another currently preferred embodiment, we disclose a CB2 binding compound of the general formula I wherein R_1 is N-OH, R_2 and R_3 are OH, and R_4 is 1,1-dimethylheptyl.

According to another currently preferred embodiment, we disclose a CB2 binding compound of the general formula I wherein R_1 is O, R_2 and R_3 are OH, and R_4 is 1,1-dimethylhept-6-ynyl.

According to another currently preferred embodiment, we now disclose a CB2 binding compound of the general formula I wherein R_1 is O, R_2 and R_3 are OH, and R_4 is 1,1-dimethyl-3-phenylpropyl.

According to another currently preferred embodiment, we disclose a CB2 binding compound of the general formula I wherein R_1 is O, R_2 and R_3 are OH, and R_4 is 1,1,3-trimethylbutyl.

According to another currently preferred embodiment, we disclose a CB2 binding compound of the general formula I wherein R_1 is O, R_2 and R_3 are OH, and R_4 is 1,1-dimethyl-p-chlorobenzyl.

According to another currently preferred embodiment, we disclose a CB2 binding compound of the general formula I wherein R_1 is O, R_2 and R_3 are OH, and R_4 is 1,1-dimethylpentyl.

According to an alternative preferred embodiment of the present invention, we disclose CB2 binding compounds having the general formula (II):

Formula II
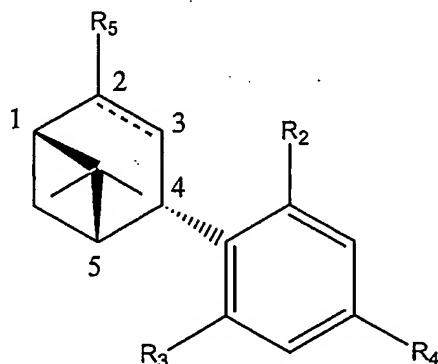
having a specific stereochemistry wherein C-5 is S, the protons at C-1 and C-5 are cis in relation to one another, the protons at C-4 and C-5 are trans, and C-2------C-3 is an optional double bond; and wherein:

R_5 is selected from the group consisting of

    (a) halogen or hydrogen,

    (b) –OR", –N(R")$_2$, –SR", –S(O)(O)NR", wherein at each occurrence R" is independently selected from the group consisting of hydrogen, C(O)R''', C(O)N(R''')$_2$, C(S)R''', saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl, C_1-C_6 alkyl-OR''', and C_1-C_6 alkyl-N(R''')$_2$, wherein at each occurrence R''' is independently

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selected from the group consisting of hydrogen or saturated or unsaturated, linear, branched or cyclic C_1-C_{12} alkyl,

(c) a saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl–SR" or C_1-C_6 alkyl–S(O)(O)NR", wherein R" is as previously defined,

(d) –S(O)R^b, –S(O)(O)R^b, –S(O)(O)OR^b wherein R^b is selected from the group consisting of hydrogen, saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl, C_1-C_6 alkyl-OR", and C_1-C_6 alkyl-N(R")$_2$, wherein at each occurrence R" is as previously defined,

(e) a saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl–S(O)R^b, C_1-C_6 alkyl–S(O)(O)R^b, C_1-C_6 alkyl–S(O)(O)OR^b wherein R^b is as previously defined, and

(f) –R^c wherein R^c is selected from the group consisting of saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl, C_1-C_6 alkyl-OR", C_1-C_6 alkyl-N(R")$_2$, C_1-C_6 alkyl-C(O)OR", and C_1-C_6 alkyl-C(O)N(R")$_2$ wherein at each occurrence R" is as previously defined;

R_2 and R_3 are each independently selected from the group consisting of

(a) halogen,

(b) –R", –OR", –N(R")$_2$, –SR", –S(O)(O)NR", wherein at each occurrence R" is as previously defined, and

(c) –S(O)R^b, –S(O)(O)R^b, –S(O)(O)OR^b wherein R^b is as previously defined; and

R_4 is selected from the group consisting of

(a) R wherein R is selected from the group consisting of hydrogen, OR'", OC(O)R'", C(O)OR'", C(O)R'", OC(O)OR'", N(R'")$_2$, NC(O)R'", NC(O)OR'", C(O)N(R'")$_2$, NC(O)N(R'")$_2$, SR'", and C(S)R'", wherein at each occurrence R'" is as previously defined,

(b) a saturated or unsaturated, linear, branched or cyclic C_1-C_{12} alkyl-R wherein R is as previously defined,

(c) an aromatic ring which can be further substituted at any position by a halogen, CN, NO_2 and R wherein R is as previously defined, and

(d) a saturated or unsaturated, linear, branched or cyclic C_1-C_{12} alkyl optionally terminated by an aromatic ring which can be further substituted as defined in (c);

with the proviso that when R_5 is R^c, then R_4 cannot be a straight or branched saturated C_1-C_{12} alkyl chain optionally preceded or interrupted by an oxygen atom and optionally substituted at the terminal carbon by a hydroxyl or a phenyl group.

According to a currently preferred embodiment, we now disclose a CB2 binding compound of the general formula II wherein R_5 is OH, R_2 and R_3 are OH, R_4 is 1,1-dimethylheptyl and there is a single bond between C-2 and C-3.

According to another currently preferred embodiment, we disclose a CB2 binding compound of the general formula II wherein R_5 is CH_3, R_2 and R_3 are OH, R_4 is 1,1-dimethylhept-6-ynyl and there is a double bond between C-2 and C-3.

The present invention also encompasses a pharmaceutical composition comprising as an active ingredient a compound of general formula (I):

Formula I

having a specific stereochemistry wherein C-5 is S, the protons at C-1 and C-5 are cis in relation to one another and the protons at C-4 and C-5 are trans; wherein:

R_1 is selected from the group consisting of

(a) O,

(b) S,

(c) $C(R')_2$ wherein each R' is independently selected from the group consisting of hydrogen, cyano, -OR", -N(R")$_2$, a saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl, C_1-C_6 alkyl-OR" or C_1-C_6 alkyl-N(R")$_2$ wherein at each occurrence R" is independently selected from the group consisting of hydrogen, C(O)R'", C(O)N(R'")$_2$, C(S)R'", saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl, C_1-C_6 alkyl-

OR''', and C_1-C_6 alkyl-N(R''')$_2$, wherein at each occurrence R''' is independently selected from the group consisting of hydrogen or saturated or unsaturated, linear, branched or cyclic C_1-C_{12} alkyl, and

(d) NR^a wherein R^a is selected from the group consisting of a hydrogen, R'' and -OR'' wherein R'' is as previously defined;

R_2 and R_3 are each independently selected from the group consisting of

(a) halogen,

(b) –R'', –OR'', –N(R'')$_2$, –SR'', –S(O)(O)NR'', wherein at each occurrence R'' is as previously defined, and

(c) –S(O)R^b, –S(O)(O)R^b, –S(O)(O)OR^b wherein R^b is selected from the group consisting of hydrogen, saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl, C_1-C_6 alkyl-OR'', and C_1-C_6 alkyl-N(R'')$_2$, wherein R'' is as previously defined; and

R_4 is selected from the group consisting of

(a) R wherein R is selected from the group consisting of hydrogen, OR''', OC(O)R''', C(O)OR''', C(O)R''', OC(O)OR''', N(R''')$_2$, NC(O)R''', NC(O)OR''', C(O)N(R''')$_2$, NC(O)N(R''')$_2$, SR''', and C(S)R''', wherein at each occurrence R''' is as previously defined,

(b) a saturated or unsaturated, linear, branched or cyclic C_1-C_{12} alkyl-R wherein R is as previously defined,

(c) an aromatic ring which can be further substituted at any position by a halogen, CN, NO$_2$ and R wherein R is as previously defined, and

(d) a saturated or unsaturated, linear, branched or cyclic C_1-C_{12} alkyl optionally terminated by an aromatic ring which can be further substituted as defined in (c).
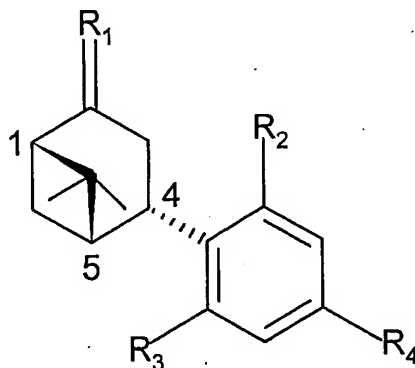
According to a currently preferred embodiment, we now disclose a pharmaceutical composition comprising as an active ingredient a compound of general formula I wherein R_1 is O, R_2 and R_3 are OH, and R_4 is 1,1-dimethylheptyl.

According to another currently preferred embodiment, we disclose a pharmaceutical composition comprising as an active ingredient a compound of the general formula I wherein R_1 is O, R_2 and R_3 are OCH$_3$, and R_4 is 1,1-dimethylheptyl.

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According to another currently preferred embodiment, we disclose a pharmaceutical composition comprising as an active ingredient a compound of the general formula I wherein R_1 is N-OH, R_2 and R_3 are OH, and R_4 is 1,1-dimethylheptyl.

According to another currently preferred embodiment, we disclose a pharmaceutical composition comprising as an active ingredient a compound of the general formula I wherein R_1 is O, R_2 and R_3 are OH, and R_4 is 1,1-dimethylhept-6-ynyl.

According to another currently preferred embodiment, we disclose a pharmaceutical composition comprising as an active ingredient a compound of the general formula I wherein R_1 is O, R_2 and R_3 are OH, and R_4 is 1,1-dimethyl-3-phenylpropyl.

According to another currently preferred embodiment, we disclose a pharmaceutical composition comprising as an active ingredient a compound of the general formula I wherein R_1 is O, R_2 and R_3 are OH, and R_4 is 1,1,3-trimethylbutyl.

According to another currently preferred embodiment, we disclose a pharmaceutical composition comprising as an active ingredient a compound of the general formula I wherein R_1 is O, R_2 and R_3 are OH, and R_4 is 1,1-dimethyl-p-chlorobenzyl.
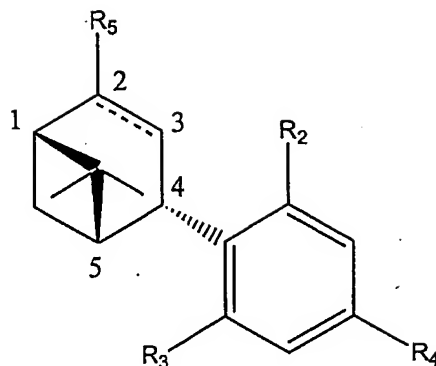
According to another currently preferred embodiment, we disclose a pharmaceutical composition comprising as an active ingredient a compound of the general formula I wherein R_1 is O, R_2 and R_3 are OH, and R_4 is 1,1-dimethylpentyl.

The present invention further encompasses a pharmaceutical composition comprising as an active ingredient a compound of general formula (II):

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Formula II

having a specific stereochemistry wherein C-5 is S, the protons at C-1 and C-5 are cis in relation to one another, the protons at C-4 and C-5 are trans, and C-2------C-3 is an optional double bond; wherein:

R_5 is selected from the group consisting of

(a) halogen or hydrogen,

(b) $-OR"$, $-N(R")_2$, $-SR"$, $-S(O)(O)NR"$, wherein at each occurrence $R"$ is independently selected from the group consisting of hydrogen, $C(O)R'''$, $C(O)N(R''')_2$, $C(S)R'''$, saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl, C_1-C_6 alkyl-OR''', and C_1-C_6 alkyl-$N(R''')_2$, wherein at each occurrence R''' is independently selected from the group consisting of hydrogen or saturated or unsaturated, linear, branched or cyclic C_1-C_{12} alkyl,

(c) a saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl$-SR"$ or C_1-C_6 alkyl$-S(O)(O)NR"$, wherein $R"$ is as previously defined,

(d) $-S(O)R^b$, $-S(O)(O)R^b$, $-S(O)(O)OR^b$ wherein R^b is selected from the group consisting of hydrogen, saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl, C_1-C_6 alkyl-$OR"$, and C_1-C_6 alkyl-$N(R")_2$, wherein at each occurrence $R"$ is as previously defined,

(e) a saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl$-S(O)R^b$, C_1-C_6 alkyl$-S(O)(O)R^b$, C_1-C_6 alkyl$-S(O)(O)OR^b$ wherein R^b is as previously defined, and

(f) $-R^c$ wherein R^c is selected from the group consisting of saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl, C_1-C_6 alkyl-$OR"$, C_1-C_6 alkyl-$N(R")_2$, C_1-C_6 alkyl-$C(O)OR"$, and C_1-C_6 alkyl-$C(O)N(R")_2$ wherein at each occurrence $R"$ is as previously defined;

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R_2 and R_3 are each independently selected from the group consisting of

    (a) halogen,

    (b) $-R''$, $-OR''$, $-N(R'')_2$, $-SR''$, $-S(O)(O)NR''$, wherein at each occurrence R'' is as previously defined, and

5   (c) $-S(O)R^b$, $-S(O)(O)R^b$, $-S(O)(O)OR^b$ wherein R^b is as previously defined; and

R_4 is selected from the group consisting of

    (a) R wherein R is selected from the group consisting of hydrogen, OR''', $OC(O)R'''$, $C(O)OR'''$, $C(O)R'''$, $OC(O)OR'''$, $N(R''')_2$, $NC(O)R'''$, $NC(O)OR'''$, $C(O)N(R''')_2$, $NC(O)N(R''')_2$, SR''', and $C(S)R'''$, wherein at each occurrence R''' is as previously

10   defined,

    (b) a saturated or unsaturated, linear, branched or cyclic C_1-C_{12} alkyl-R wherein R is as previously defined,

    (c) an aromatic ring which can be further substituted at any position by a halogen, CN, NO_2 and R wherein R is as previously defined, and

15   (d) a saturated or unsaturated, linear, branched or cyclic C_1-C_{12} alkyl optionally terminated by an aromatic ring which can be further substituted as defined in (c);

    with the proviso that when R_5 is R^c, then R_4 cannot be a straight or branched saturated C_1-C_{12} alkyl chain optionally preceded or interrupted by an oxygen atom and optionally substituted at the terminal carbon by a hydroxyl or a phenyl group.

20   According to a currently preferred embodiment, we now disclose a pharmaceutical composition comprising as an active ingredient a compound of general formula II wherein R_5 is OH, R_2 and R_3 are OH, R_4 is 1,1-dimethylheptyl and there is a single bond between C-2 and C-3.

    According to another currently preferred embodiment, we disclose a pharmaceutical

25 composition comprising as an active ingredient a compound of the general formula II wherein R_5 is CH_3, R_2 and R_3 are OH, and R_4 is 1,1-dimethylhept-6-ynyl and there is a double bond between C-2 and C-3.

    The novel compositions may contain in addition to the active ingredient conventional pharmaceutically acceptable carriers, diluents and excipients necessary to produce a

30 physiologically acceptable and stable formulation.

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The pharmaceutical compositions can be administered by any conventional and appropriate route including oral, parenteral, intravenous, intramuscular, intralesional, subcutaneous, transdermal, intrathecal, rectal or intranasal.

A further aspect of the present invention provides a method of treating a patient by
5   stimulating CB2 receptors, which comprises administering to said patient a pharmaceutical composition comprising a therapeutically effective amount of a compound of general formulae I and II according to the present invention.

Accordingly, the present invention provides novel compounds of the general formulae I and II that are useful for immunomodulation and therapies for indications amenable to CB2
10 receptor modulation including but not limited to: autoimmune diseases including rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosis, myasthenia gravis, diabetes mellitus type I, and psoriasis and immune related disorders including but not limited to tissue rejection in organ transplants, malabsorption syndromes such as celiac disease, pulmonary diseases such as asthma and Sjögren's syndrome, inflammation including inflammatory
15 bowel disease, pain including peripheral, neuropathic and referred pain, muscle spasticity, cardiovascular disorders including arrhythmia, hypertension and myocardial ischemia, neurological disorders including stroke, migraine and cluster headaches, neurodegenerative diseases including Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, Huntington's chorea, prion–associated neurodegeneration, CNS poisoning and certain types
20 of cancer.

The present invention encompasses the use of the compounds of the general formulae I and II for the preparation of a medicament for the treatment and prevention autoimmune diseases including but not limited to rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosis, myasthenia gravis, diabetes mellitus type I, psoriasis and immune related
25 disorders including but not limited to tissue rejection in organ transplants, malabsorption syndromes such as celiac disease, pulmonary diseases such as asthma and Sjögren's syndrome, inflammation including inflammatory bowel disease, pain including peripheral, neuropathic and referred pain, muscle spasticity, cardiovascular disorders including arrhythmia, hypertension and myocardial ischemia, neurological disorders including stroke,
30 migraine and cluster headaches, neurodegenerative diseases including Parkinson's disease,

Alzheimer's disease, amyotrophic lateral sclerosis, Huntington's chorea, prion–associated neurodegeneration, CNS poisoning and certain types of cancer, as shown in the specification.

While the compounds and compositions of the present invention are specifically designed to serve as ligands of the peripheral cannabinoid receptor CB2, they may also possess other desirable therapeutic attributes of the class of compounds referred to as "non-classical cannabinoids" whether or not mediated via the CB2 receptor. Thus the compounds and compositions of general formulae I and II have neuroprotective properties in addition to their immunomodulatory activity.

As exemplified herein below, we have now found that the known CB2 specific agonist HU-308, (WO 01/32169), is not only effective in the treatment of peripheral pain but also in the treatment of neuropathic pain. Moreover, we have now found that HU-308 is particularly effective in the treatment and the prevention of Parkinson's disease.

Moreover, we have recently found that some of the novel preferred CB2 binding compounds, including one previously disclosed in international patent application WO 01/32169, are effective in modulating gene expression of mediators involved in inflammation such as cytokines, chemokines and enzymes like COX-2.

BRIEF DESCRIPTION OF THE FIGURES

To assist in the understanding of the invention and in particular of the data that are given in the Examples, the following drawing figures are presented herein:

Figure 1 shows the binding of selected bicyclic compounds to the CB1 and CB2 human cannabinoid receptors.

Figure 2 shows the effect of selected bicyclic compounds of formulae I and II on IL-1β secretion from activated macrophages.

Figure 3 shows the effect of compound A, (-)-4-[4-(1,1-Dimethyl-heptyl)-2,6-dihydroxy-phenyl]-6,6-dimethyl-bicyclo[3.1.1]heptan-2-one, and compound K, (-)-5-(1,1-Dimethyl-

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pentyl)-2-(4,6,6-trimethyl-bicyclo[3.1.1]hept-3-en-2-yl)-benzene-1,3-diol, at various doses on IL-2 secretion from activated T cells.

Figure 4 shows the effect of compounds A and K, at various doses, in the EAE model for multiple sclerosis.

5   Figure 5 shows the effect of the CB2 agonist HU-308, and compounds A and K, at various doses, in the DTH model for allergic or other immune reactions.

Figure 6 shows the effect of the CB2 agonist HU-308 in the MPTP model for Parkinson's disease.

Figure 7 shows the effect of the compound A at two doses in the Constriction Nerve Injury
10   model for Chronic Neuropathic pain.

Figure 8 shows the effect of compounds A and K, at various doses, in the Tail Flick model for Acute Peripheral pain. Panel A presents the results obtained 30 minutes after treatment and panel B the results obtained 90 minutes after treatment.

15   **DETAILED DESCRIPTION OF THE INVENTION**

The present invention provides novel compounds belonging to the non-classical cannabinoids, as well as pharmaceutical compositions comprising these compounds, and methods of using such compounds and compositions. The compounds of this class show affinity for cannabinoid receptors. The preferred novel compounds of this invention show
20   affinity for the peripheral human cannabinoid receptor, CB2. The compositions of the present invention have been shown to possess immunomodulatory, anti-inflammatory, analgesic, neuroprotective and certain anti-tumoral properties. The action of some compounds may result in modulation of transcription of genes involved in immunomodulation and inflammation.

25   By virtue of their anti-inflammatory and immunomodulatory properties, it will be recognized that the compositions according to the present invention will be useful for treating

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indications having an inflammatory or autoimmune mechanism involved in their etiology or pathogenesis exemplified by arthritis, including rheumatoid arthritis, arthritis, multiple sclerosis, systemic lupus erythematosus (SLE), myasthenia gravis, diabetes mellitus type I and psoriasis, immune related disorders including but not limited to tissue rejection in organ transplants, malabsorption syndromes such as celiac, pulmonary diseases such as asthma and Sjögren's syndrome, inflammatory bowel disease, and rheumatic diseases.

While the compounds and compositions of the present invention were designed to be CB2 ligands they share other properties of this class of non-classical cannabinoids including neuroprotective properties (US Patent 5,434,295). By virtue of their neuroprotective properties, it will be recognized that the compositions according to the present invention will be useful in treating neurological disorders including but not limited to stroke, migraine, and cluster headaches. The composition of the present invention may also be effective in treating certain chronic degenerative diseases that are characterized by gradual selective neuronal loss. In this connection, the compositions of the present invention are contemplated as therapeutically effective in the treatment of Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, Huntington's chorea and prion-associated neurodegeneration. Neuroprotection conferred by CB2 agonists could also be effective in protection and/or treatment of neurotoxic agents, such as nerve gas, as well as other insults to brain or nervous tissue by way of chemical or biological agents.

By virtue of their analgesic properties it will be recognized that the compositions according to the present invention will be useful in treating pain including peripheral, neuropathic and referred pain. The compositions of the present invention may also be effective in cardioprotection from arrhythmia, hypertension and myocardial ischemia. The compositions of the present invention may also be effective in the treatment of muscle spasm and tremor.

Another feature of the present invention is the ability of the disclosed compounds to prevent or treat certain cancers, including malignant brain tumors, where CB2 binding ligands may trigger apoptosis.

Moreover, we have found that some of our preferred CB2 binding compounds, including a compound disclosed in international patent application WO 01/32169, are

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effective in modulating the transcription of genes involved in immunomodulation and inflammation.

Furthermore, we now disclose that the known CB2 specific agonist HU-308 that was found to be effective in the treatment of peripheral pain is unexpectedly also effective in the
5   treatment of neuropathic pain as assessed by chronic constriction of the sciatic nerve in rodent models.

Additionally, it was also discovered that HU-308 reduced significantly the extent of cell death produced in the Substantia Nigra of mice treated with the neurotoxin MPTP. This suggests that this compound may prove especially effective in the treatment of Parkinson's
10   disease.

Bicyclic compounds shown to have high affinity and specificity for the CB2 receptor have been disclosed by Makriyannis and co-worker in international patent application WO 01/28497. A person skilled in the art would discern in that disclosure that the compounds disclosed are of opposite stereochemistry to those of the present invention, since the dimethyl
15   of the four member ring is below the plane of the terpenic ring while the aryl group lies above this same plane, as drawn in formulae I and II of that disclosure. According to the nomenclature adopted in the present disclosure, the Makriyannis' compounds are of stereochemical orientation wherein C-1, C-4 and C-5 are R. In general, it has been possible to functionally differentiate between the R and S enantiomers of cannabinoid and cannabinoid-
20   related compounds. The compounds HU-210 and HU-211 exemplify this. HU-210 is the (-)(3R,4R) enantiomer of the synthetic cannabinoid, 7-hydroxy-Δ^6-tetrahydrocannabinol-1,1-dimethylheptyl. HU-211 is the (+)(3S,4S) enantiomer of that compound. In contrast to HU-210, HU-211 exhibits low affinity to the cannabinoid receptors and is thus non-psychotropic. In addition, it functions as a noncompetitive NMDA-receptor antagonist and as a
25   neuroprotective agent, two properties absent in HU-210 (US Patent 5,284,867).

The inventors of the present invention have unexpectedly found that the enantiomers of stereochemistry opposite to the compounds disclosed in WO 01/28497 are effective CB2 receptor ligands. The present disclosure teaches novel derivatives of (+) α-pinene wherein C-5 is S, the protons at C-1 and C-5 are cis in relation to one another and the protons at C-4 and

C-5 are trans, as depicted in formulae I-III. Moreover, the present invention relates to the use of these novel CB2 ligands for the preparation of compounds to prevent or treat autoimmune diseases and related disorders, inflammation, pain, muscle spasticity, cardiovascular disorders, neurological disorders, neurodegenerative diseases, CNS poisoning and certain

5   types of cancer.

In the present invention we will refer to the following numbering of positions in the ring structure, where positions 1, 4 and 5 are chiral centers. The stereochemistry of the compounds disclosed in the present invention is such that C-5 is S, the protons at C-1 and C-5 are cis in relation to one another and the protons at C-4 and C-5 are trans as shown in formula

10   (III):

Formula III
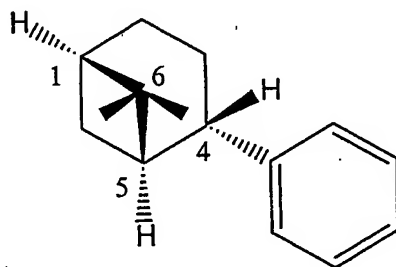
In the present invention, binding affinity is represented by the IC_{50} value, namely the concentration of an analog that will displace 50% of a radiolabeled agonist from the CB

15   receptors. "CB2 specific" denotes compounds with a ratio of CB2/CB1 binding affinity that is at least 10, preferably 20, more preferably 50 and most preferably 100 or greater. Preferably these ratios will be obtained for human CB1 and CB2 receptors.

Throughout this specification, certain compounds of the present invention may be referred to by capital letters rather than by their full chemical names. The alkyl substituents

20   can be saturated or unsaturated, linear, branched or cyclic, the latter only when the number of carbon atoms in the alkyl chain is greater than or equal to three. OC(O)R represents esters, OC(O)NR carbamates, OC(S)R thioesters, NR_2 amines, NRC(O)R amides, NRC(O)NR ureas, NRC(S)R thioamides, SR thiols or sulfides, S(O)R sulfoxides, SC(O)R thioesters, SC(O)NR thiocarbamates, SC(S)R dithioesters, S(O)(O)R sulfones, S(O)(O)OR sulfonates, S(O)(O)NR

sulfonamides,   S(O)(O)NC(O)R   acylsulfonamides,   S(O)(O)NC(O)NR   sulfonurea,
S(O)(O)NC(S)R thioacylsulfonamide when R is a hydrogen or an alkyl chain.

The present invention relates to CB2 binding compounds of the general formula (I):

Formula I

5   having a specific stereochemistry wherein C-5 is S, the protons at C-1 and C-5 are cis in relation to one another and the protons at C-4 and C-5 are trans; wherein:
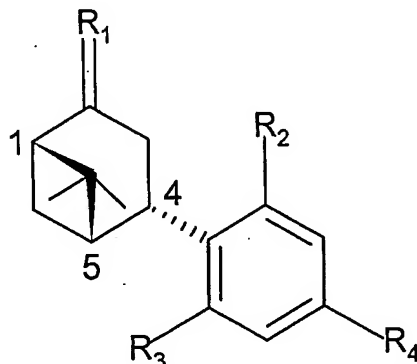
R_1 is selected from the group consisting of

   (a) O,

10   (b) S,

   (c) $C(R')_2$ wherein each R' is independently selected from the group consisting of hydrogen, cyano, -OR", $-N(R'')_2$, a saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl, C_1-C_6 alkyl-OR" or C_1-C_6 alkyl-$N(R'')_2$ wherein at each occurrence R" is independently selected from the group consisting of hydrogen, C(O)R''', $C(O)N(R''')_2$,

15   C(S)R''', saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl, C_1-C_6 alkyl-OR''', and C_1-C_6 alkyl-$N(R''')_2$, wherein at each occurrence R''' is independently selected from the group consisting of hydrogen or saturated or unsaturated, linear, branched or cyclic C_1-C_{12} alkyl, and

   (d) NR^a wherein R^a is selected from the group consisting of a hydrogen, R" and -OR"

20   wherein R" is as previously defined;

R_2 and R_3 are each independently selected from the group consisting of

   (a) halogen,

   (b) –R", –OR", $–N(R'')_2$, –SR", –S(O)(O)NR", wherein at each occurrence R" is as previously defined, and

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(c) $-S(O)R^b$, $-S(O)(O)R^b$, $-S(O)(O)OR^b$ wherein R^b is selected from the group consisting of hydrogen, saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl, C_1-C_6 alkyl-OR", and C_1-C_6 alkyl-N(R")$_2$, wherein R" is as previously defined; and

5   R_4 is selected from the group consisting of

(a) R wherein R is selected from the group consisting of hydrogen, OR''', OC(O)R''', C(O)OR''', C(O)R''', OC(O)OR''', N(R''')$_2$, NC(O)R''', NC(O)OR''', C(O)N(R''')$_2$, NC(O)N(R''')$_2$, SR''', and C(S)R''', wherein at each occurrence R''' is as previously defined,

10   (b) a saturated or unsaturated, linear, branched or cyclic C_1-C_{12} alkyl-R wherein R is as previously defined,

(c) an aromatic ring which can be further substituted at any position by a halogen, CN, NO$_2$ and R wherein R is as previously defined, and

(d) a saturated or unsaturated, linear, branched or cyclic C_1-C_{12} alkyl optionally

15   terminated by an aromatic ring which can be further substituted as defined in (c).

According to a currently preferred embodiment, we now disclose a CB2 binding compound of the general formula I wherein R_1 is O, R_2 and R_3 are OH, and R_4 is 1,1-dimethylheptyl.

According to another currently preferred embodiment, we disclose a CB2 binding
20   compound of the general formula I wherein R_1 is O, R_2 and R_3 are OCH$_3$, and R_4 is 1,1-dimethylheptyl.

According to another currently preferred embodiment, we disclose a CB2 binding compound of the general formula I wherein R_1 is N-OH, R_2 and R_3 are OH, and R_4 is 1,1-dimethylheptyl.

25   According to another currently preferred embodiment, we disclose a CB2 binding compound of the general formula I wherein R_1 is O, R_2 and R_3 are OH, and R_4 is 1,1-dimethylhept-6-ynyl.

According to another currently preferred embodiment, we disclose a CB2 binding compound of the general formula I wherein R_1 is O, R_2 and R_3 are OH, and R_4 is 1,1-dimethyl-3-phenylpropyl.

According to another currently preferred embodiment, we disclose a CB2 binding compound of the general formula I wherein R_1 is O, R_2 and R_3 are OH, and R_4 is 1,1,3-trimethylbutyl.

According to another currently preferred embodiment, we disclose a CB2 binding compound of the general formula I wherein R_1 is O, R_2 and R_3 are OH, and R_4 is 1,1-dimethyl-p-chlorobenzyl.

According to another currently preferred embodiment, we disclose a CB2 binding compound of the general formula I wherein R_1 is O, R_2 and R_3 are OH, and R_4 is 1,1-dimethylpentyl.

The present invention further relates to CB2 binding compounds of the general formula (II):

Formula II

having a specific stereochemistry wherein C-5 is S, the protons at C-1 and C-5 are cis in relation to one another, the protons at C-4 and C-5 are trans, and C-2------C-3 is an optional double bond; wherein:

R_5 is selected from the group consisting of

    (a) halogen or hydrogen,

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(b) $-OR''$, $-N(R'')_2$, $-SR''$, $-S(O)(O)NR''$, wherein at each occurrence R'' is independently selected from the group consisting of hydrogen, $C(O)R'''$, $C(O)N(R''')_2$, $C(S)R'''$, saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl, C_1-C_6 alkyl-OR''', and C_1-C_6 alkyl-$N(R''')_2$, wherein at each occurrence R''' is independently selected from the group consisting of hydrogen or saturated or unsaturated, linear, branched or cyclic C_1-C_{12} alkyl,

(c) a saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl–SR'' or C_1-C_6 alkyl–$S(O)(O)NR''$, wherein R'' is as previously defined,

(d) $-S(O)R^b$, $-S(O)(O)R^b$, $-S(O)(O)OR^b$ wherein R^b is selected from the group consisting of hydrogen, saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl, C_1-C_6 alkyl-OR'', and C_1-C_6 alkyl-$N(R'')_2$, wherein at each occurrence R'' is as previously defined,

(e) a saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl–$S(O)R^b$, C_1-C_6 alkyl–$S(O)(O)R^b$, C_1-C_6 alkyl–$S(O)(O)OR^b$ wherein R^b is as previously defined, and

(f) $-R^c$ wherein R^c is selected from the group consisting of saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl, C_1-C_6 alkyl-OR'', C_1-C_6 alkyl-$N(R'')_2$, C_1-C_6 alkyl-$C(O)OR''$, and C_1-C_6 alkyl-$C(O)N(R'')_2$ wherein at each occurrence R'' is as previously defined;

R_2 and R_3 are each independently selected from the group consisting of

(a) halogen,

(b) $-R''$, $-OR''$, $-N(R'')_2$, $-SR''$, $-S(O)(O)NR''$, wherein at each occurrence R'' is as previously defined, and

(c) $-S(O)R^b$, $-S(O)(O)R^b$, $-S(O)(O)OR^b$ wherein R^b is as previously defined; and

R_4 is selected from the group consisting of

(a) R wherein R is selected from the group consisting of hydrogen, OR''', $OC(O)R'''$, $C(O)OR'''$, $C(O)R'''$, $OC(O)OR'''$, $N(R''')_2$, $NC(O)R'''$, $NC(O)OR'''$, $C(O)N(R''')_2$, $NC(O)N(R''')_2$, SR''', and $C(S)R'''$, wherein at each occurrence R''' is as previously defined,

(b) a saturated or unsaturated, linear, branched or cyclic C_1-C_{12} alkyl-R wherein R is as previously defined,

(c) an aromatic ring which can be further substituted at any position by a halogen, CN, NO_2 and R wherein R is as previously defined, and

(d) a saturated or unsaturated, linear, branched or cyclic C_1-C_{12} alkyl optionally terminated by an aromatic ring which can be further substituted as defined in (c);

with the proviso that when R_5 is R^c, then R_4 cannot be a straight or branched saturated C_1-C_{12} alkyl chain optionally preceded or interrupted by an oxygen atom and optionally substituted at the terminal carbon by a hydroxyl or a phenyl group.

According to a currently preferred embodiment, we now disclose a CB2 binding compound of the general formula II wherein R_5 is OH, R_2 and R_3 are OH, R_4 is 1,1-dimethylheptyl and there is a single bond between C-2 and C-3.

According to another currently preferred embodiment, we disclose a CB2 binding compound of the general formula II wherein R_5 is CH_3, R_2 and R_3 are OH, R_4 is 1,1-dimethylhept-6-ynyl and there is a double bond between C-2 and C-3.

The present invention relates to pharmaceutical compositions for the purposes set out above, comprising as an active ingredient a compound of the general formula (I):
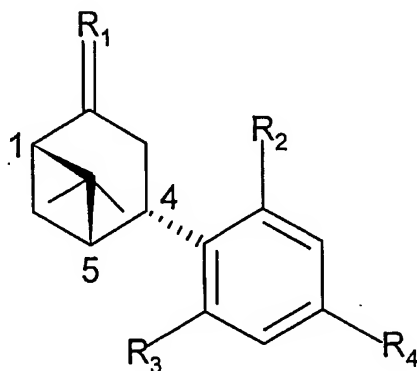
Formula I

having a specific stereochemistry wherein C-5 is S, the protons at C-1 and C-5 are cis in relation to one another and the protons at C-4 and C-5 are trans; and wherein the substituents are as defined above for formula I.

According to a currently preferred embodiment, we now disclose a pharmaceutical composition comprising as an active ingredient a compound of general formula I wherein R_1 is O, R_2 and R_3 are OH, and R_4 is 1,1-dimethylheptyl.

According to another currently preferred embodiment, we disclose a pharmaceutical composition comprising as an active ingredient a compound of the general formula I wherein R_1 is O, R_2 and R_3 are OCH_3, and R_4 is 1,1-dimethylheptyl.

According to another currently preferred embodiment, we disclose a pharmaceutical composition comprising as an active ingredient a compound of the general formula I wherein R_1 is N-OH, R_2 and R_3 are OH, and R_4 is 1,1-dimethylheptyl.

According to another currently preferred embodiment, we disclose a pharmaceutical composition comprising as an active ingredient a compound of the general formula I wherein R_1 is O, R_2 and R_3 are OH, and R_4 is 1,1-dimethylhept-6-ynyl.

According to another currently preferred embodiment, we disclose a pharmaceutical composition comprising as an active ingredient a compound of the general formula I wherein R_1 is O, R_2 and R_3 are OH, and R_4 is 1,1-dimethyl-3-phenylpropyl.

According to another currently preferred embodiment, we disclose a pharmaceutical composition comprising as an active ingredient a compound of the general formula I wherein R_1 is O, R_2 and R_3 are OH, and R_4 is 1,1,3-trimethylbutyl.

According to another currently preferred embodiment, we disclose a pharmaceutical composition comprising as an active ingredient a compound of the general formula I wherein R_1 is O, R_2 and R_3 are OH, and R_4 is 1,1-dimethyl-p-chlorobenzyl.

According to another currently preferred embodiment, we disclose a pharmaceutical composition comprising as an active ingredient a compound of the general formula I wherein R_1 is O, R_2 and R_3 are OH, and R_4 is 1,1-dimethylpenthyl.

The present invention further relates to pharmaceutical compositions for the purposes set out above comprising as an active ingredient a compound of the general formula (II):

Formula II

having a specific stereochemistry wherein C-5 is S, the protons at C-1 and C-5 are cis in relation to one another, the protons at C-4 and C-5 are trans and C-2------C-3 is an optional double bond; and wherein the substituents are as defined above for formula II.

According to a currently preferred embodiment, we disclose a pharmaceutical composition comprising as an active ingredient a compound of the general formula II wherein R_5 is OH, R_2 and R_3 are OH, R_4 is 1,1-dimethylheptyl and there is a single bond between C-2 and C-3.
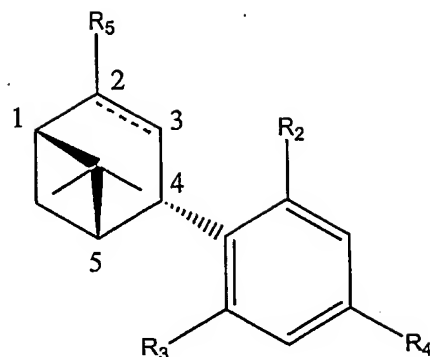
According to another currently preferred embodiment, we disclose a pharmaceutical composition comprising as an active ingredient a compound of the general formula II wherein R_5 is CH_3, R_2 and R_3 are OH, R_4 is 1,1-dimethylhept-6-ynyl and there is a double bond between C-2 and C-3.

The novel non-classical cannabinoids according to the present invention most preferably bind efficiently to the CB2 receptor but weakly to CB1 receptor, the latter known to mediate the psychotropic activity in the CNS.

The present invention further relates to new therapies utilizing the compositions of the present invention for the prevention and treatment of autoimmune diseases including rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosis, myasthenia gravis, diabetes mellitus type I and psoriasis and immune related disorders including but not limited to tissue rejection in organ transplants, malabsorption syndromes such as celiac disease, pulmonary diseases such as asthma and Sjögren's syndrome, inflammation including inflammatory bowel disease, pain including peripheral, neuropathic and referred pain, muscle

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spasticity, cardiovascular disorders including arrhythmia, hypertension and myocardial ischemia, neurological disorders including stroke, migraine and cluster headaches, neurodegenerative diseases including Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, Huntington's chorea, prion-associated neurodegeneration, CNS poisoning

5   and certain types of cancer.

The novel compositions contain, in addition to the active ingredient, conventional pharmaceutically acceptable carriers, diluents and excipients necessary to produce a physiologically acceptable and stable formulation. For compounds having solubility problems, and some compounds of the present invention are characteristically hydrophobic

10   and practically insoluble in water with high lipophilicity, as expressed by their high octanol/water partition coefficient and log P values, formulation strategies to prepare acceptable dosage forms will be applied. Enabling therapeutically effective and convenient administration of the compounds of the present invention is an integral part of this invention.

For water soluble compounds standard formulations will be utilized. Solid

15   compositions for oral administration such as tablets, pills, capsules or the like may be prepared by mixing the active ingredient with conventional, pharmaceutically acceptable ingredients such as corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate and gums with pharmaceutically acceptable diluents. The tablets or pills can be coated or otherwise compounded with pharmaceutically acceptable

20   materials known in the art to provide a dosage form affording prolonged action or sustained release. Other solid compositions can be prepared as suppositories, for rectal administration. Liquid forms may be prepared for oral administration or for injection, the term including but not limited to subcutaneous, transdermal, intravenous, intrathecal, intralesional, adjacent to or into tumors, and other parenteral routes of administration. The liquid compositions include

25   aqueous solutions, with or without organic cosolvents, aqueous or oil suspensions including but not limited to cyclodextrins as suspending agent, flavored emulsions with edible oils, as well as elixirs and similar pharmaceutical vehicles. In addition, the compositions of the present invention may be formed as aerosols, for intranasal and like administration. Topical pharmaceutical compositions of the present invention may be formulated as an aqueous

30   solution, lotion, gel, cream, ointment, emulsion or adhesive film with pharmaceutically acceptable excipients including but not limited to propylene glycol, lecithin base, aloe vera, petrolatum or other such excipients as are known in the art.

Prior to their use as medicaments, the pharmaceutical compositions will generally be formulated in unit dosage. The active dose for humans is generally in the range of from 0.05 mg to about 50 mg per kg body weight, in a regimen of 1-4 times a day. The preferred range of dosage is from 0.1 mg to about 20 mg per kg body weight. However, it is evident to the

5   man skilled in the art that dosages would be determined by the attending physician, according to the disease to be treated, the method of administration, the patient's age, weight, contraindications and the like.

The principles of the present invention will be more fully understood in the following examples, which are to be construed in a non-limitative manner.

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EXAMPLES

SYNTHETIC EXAMPLES

Synthesis of compound A: (-)-4-[4-(1,1-Dimethyl-heptyl)-2,6-dihydroxy-phenyl]-6,6-dimethyl-bicyclo[3.1.1]heptan-2-one.

15   The synthesis of compound A is depicted in Scheme 1 when R is 1,1-dimethylheptyl.

To a 3-necked flask containing n-butyl lithium (196 ml, 2M) and 44 g potassium tert-butoxide at -78°C under nitrogen atmosphere, 50 ml of (+)-α-pinene (1) is added dropwise. The reaction is allowed to warm up to room temperature and is stirred continuously for 48 hours. The reaction is then cooled to −78°C. Trimethyl borate (113 ml) in 80 ml of ether is added and

20   the reaction is allowed to warm up to room temperature and is stirred for one additional hour. The organic layer is separated, and the aqueous layer is extracted with n-hexane (3 x 80 ml). The combined organic phases are washed with brine, dried over anhydrous sodium sulfate, filtered and evaporated to dryness to afford compound (2), (+)-β-pinene. This procedure is according to Brown et al. (Brown H.C. et al., J. Org. Chem. 54: 1764-6, 1989).

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Scheme 1

(+)-α-pinene (1)   (+)-β-pinene (2)   (-)-Nopinone (3):

(+)-Nopinone enol acetate (4)   (5)   (6)
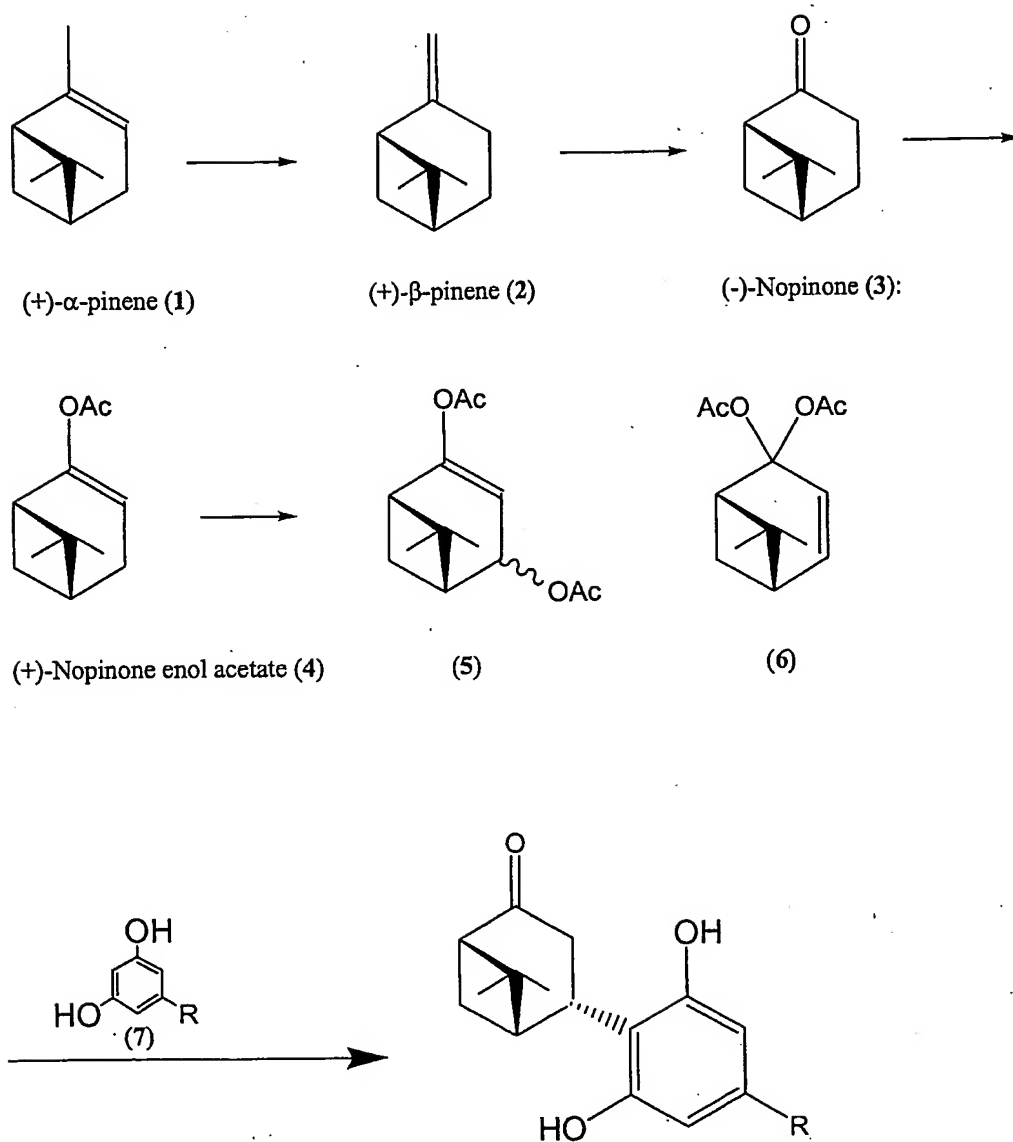
compound A: R=1,1-Dimethylheptyl
compound L: R=1,1-Dimethylpentyl

To (+)-β-pinene (2) (30.8 g) are added RuCl₃ (0.470 g), benzyltributyl ammonium chloride
5   (2.12 g) dissolved in 250 ml of ethyl acetate. To this mixture, sodium periodate (145.5 g) in
1.3 L of water is added dropwise, stirred at room temperature for 3 hours and left overnight.
250 ml of ethyl acetate are added to the reaction mixture. The organic phase is separated,

washed with 500 ml of brine, 500 ml of 10% sodium sulfite, dried over anhydrous sodium

sulfate, filtered, evaporated in vacuum to afford compound (3), (-)-Nopinone. This procedure

is according to Yuasa et al. (Yuasa Y. et al., J. Essent. Oil. Res. 10: 39-42, 1998). (-)-

Nopinone (3) (14.86 g) and p-toluenesulfonic acid (1.48 g) are dissolved in isoprenyl acetate

5   (148 ml). The reaction mixture is heated at reflux for 5 hours using a Dean-Stark apparatus to

remove the acetone. The solvents are removed under vacuum, and the residue is taken in 400

ml of ether, washed with water, dried over anhydrous sodium sulfate, filtered and evaporated

to afford compound (4), (+)-Nopinone enol acetate. This procedure is based on a method

developed for the opposite enantiomer by Archer et al. (Archer R.A. et al., J. Org. Chem. 42:

10 2277-84, 1977). To a solution of 16.17 g of (+)-Nopinone enol acetate (4) in 202 ml of dry

toluene are added 62.2 g of Pb(OAc)₄ (previously dried in vacuo over P_2O_5/KOH overnight).

The reaction mixture is heated at 80°C for 3.5 hours, cooled, filtered, washed with saturated

sodium bicarbonate. The organic layer is separated, dried over anhydrous sodium sulfate and

evaporated under vacuum to yield (+)-6,6-Dimethyl-2,4-diacetoxy-2-norpinene (5) and (-)-

15 6,6-dimethyl-2,2-diacetoxy-3-norpinene (6). A mixture of 5 and 6 (1.18 g, 5 mmol),

resorcinol wherein R is 1,1-dimethylheptyl (7) (1.18 g, 5 mmol) and p-toluenesulfonic acid

(0.95 g, 5 mmol) in chloroform (50 ml) is allowed to react at room temperature for 4 hours.

Ether (30 ml) is then added, and the organic phase is washed with saturated sodium

bicarbonate, water, then dried over anhydrous sodium sulfate, filtered and evaporated. The

20 residue is allowed to crystallize in acetonitrile to provide 0.5 g of crystals. The mother liquors

are chromatographed over silica gel to afford further 0.7 g of pure compound A.

Synthesis of compound L: (-)-4-[4-(1,1-Dimethyl-pentyl)-2,6-dihydroxy-phenyl]-6,6-
dimethyl-bicyclo[3.1.1]heptan-2-one.

The synthesis of compound L is depicted in Scheme 1 when the R moiety of the resorcinol

25 compound is 1,1-dimethylpentyl. Compounds 1 to 6 were prepared as described for the

synthesis of compound A.

Synthesis of compound B: (-)-4-[4-(1,1-Dimethyl-heptyl)-2,6-dimethoxyphenyl]-6,6-
dimethyl- bicyclo[3.1.1]heptan-2-one.

The synthesis of compound B is depicted in Scheme 2.

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Scheme 2.

compound A   compound B
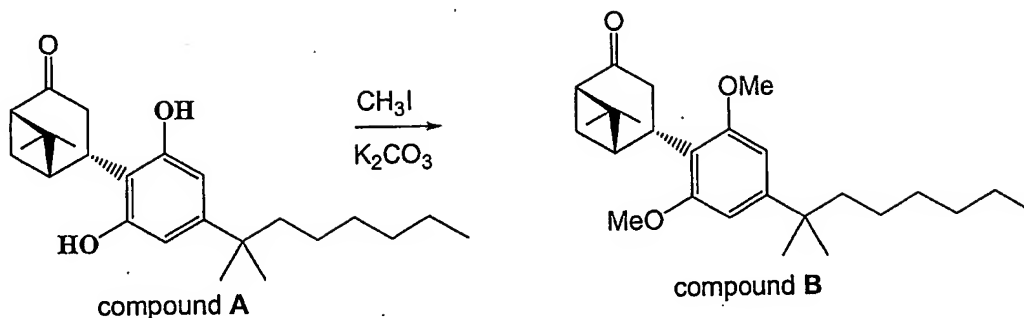
To a solution of compound A (115 mg, 0.3 mmol) in DMF (5 ml) was added potassium carbonate (0.5 g, 3.6 mmol) and the mixture was stirred for 10 min Iodomethane (0.15 ml, 24

5   mmol) was then added and the mixture is stirred overnight at room temperature. Water was added to the reaction mixture and extracted with EtOAc. The organic phase is washed twice with water, dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed over reversed phase C-18 silica gel column using 10% water in acetonitrile as the eluent to afford 98 mg of compound B.

10   **Synthesis of compound C:** (-)-5-(1,1-Dimethyl-heptyl)-2-(4-hydroxy-6,6-dimethylbicyclo [3.1.1] hept-2-yl)-benzene-1,3-diol.

The synthesis of compound C is depicted in Scheme 3.

Scheme 3.

compound A   compound C
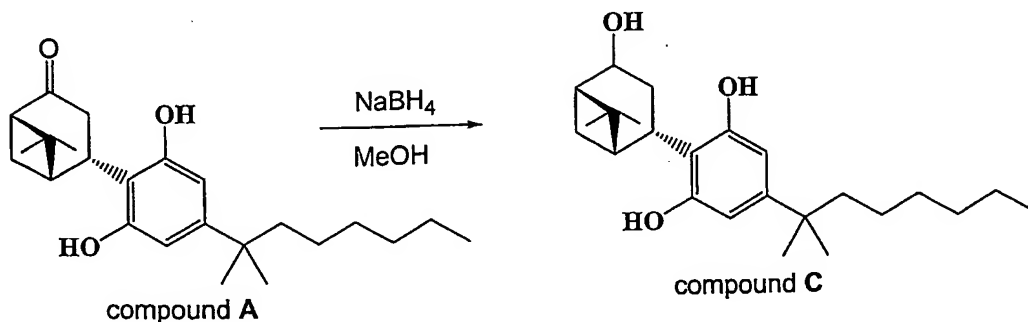
15   100 mg of compound A dissolved in 10 ml of methanol were cooled to 0°C. Sodium borohydride (200 mg) was added portionwise and the reaction mixture was stirred for 4 hours. The mixture was poured into 50 ml of 5% HCl, extracted with ethyl acetate (2 x 30 ml), dried

over Na_2SO_4, filtered and evaporated to give 90 mg of compound C in the form of white powder.

Synthesis of compound D: (-)-4-[4-(1,1-Dimethyl-heptyl)-2,6-dihydroxy-phenyl]-6,6-dimethyl- bicyclo[3.1.1]heptan-2-one oxime.

5   The synthesis of compound D is depicted in Scheme 4.
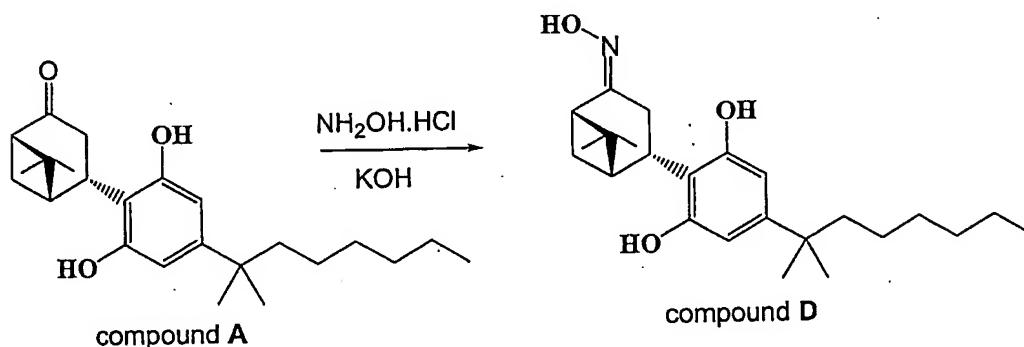
Scheme 4.

compound A   compound D

Hydroxylamine hydrochloride (37.3 mg) was dissolved in 5 ml of water and the solution
10   cooled to 0°C. Potassium hydroxide (30 mg) in 1 ml of water was added slowly. Compound A (372.5 mg) was added followed by addition of methanol to dissolve all the components. After 3 hours of stirring, no starting material could be observed. Water was then added and the solution was extracted with ethyl acetate, dried over Na_2SO_4, filtered and evaporated to afford 380 mg of compound D.

15   **Synthesis of compound E:** 5-(1,1-Dimethyl-hept-6-ynyl)-2-(4,6,6-trimethyl-bicyclo [3.1.1]hept-3-en-2-yl)-benzene-1,3-diol.

The synthesis of compound E is depicted in Scheme 5.

The reaction was carried out under anhydrous conditions. A well-stirred mixture of (+) verbenol (0.505 g, 3.3 mmol), 3-(1,1-dimethylhept-6-ynyl) resorcinol (0.77g, 3.3 mmol) and
20   catalytic amount of anhydrous p-toluenesulfonic acid in dry dicholoromethane (10 ml) were stirred at room temperature for 2 hours. The mixture was poured onto an aqueous solution of sodium bicarbonate (50 ml) and the aqueous phase was extracted with dichloromethane (3 x 30 ml). The combined organic layers were then washed with water (3 x 30 ml), and brine (3 x

100 ml). The organic layer was dried over Na$_2$SO$_4$, filtered and evaporated. The crude material thus obtained was purified by flash chromatography on silica gel using 5% ether/ petroleum ether as the eluent to afford 1.034 g of compound E.

Scheme 5.

(+) verbenol   1,1-dimethylhept-6-ynyl resorcinol   compound **E**
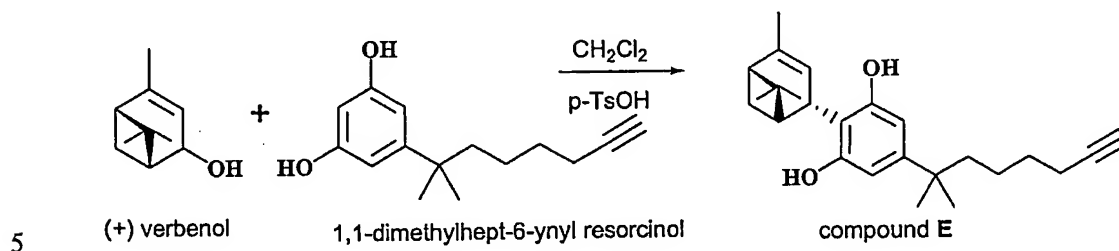
The synthesis of compound F: (-)-4-[4-(1,1-Dimethyl-hept-6-ynyl)-2,6-dihydroxy-phenyl]-6,6-dimethyl- bicyclo[3.1.1]heptan-2-one.

The synthesis of compound F is depicted in Scheme 6.

(3,5-Dimethoxyphenyl)-N-methoxy-N-methylcarboxamide **(8)** was prepared as described by Harrington et al. (Harrington P.E. et al., J. Org. Chem. 65: 6576-82, 2000). 1-(trimethylsilyl)-6-bromo-1-hexyne **(9)** was prepared according to Negishi et al. (Negishi E-I et al., J. Amer. Chem. Soc. 110: 5383-96, 1988). [7-(3,5-Dimethoxyphenyl)-7-oxo-1-heptynyl] trimethyl silane **(10)** was prepared according to the following procedure. To magnesium metal (300 mg) in 5 ml of anhydrous THF, a catalytic amount of dibromomethane was added and the reaction mixture was heated to reflux for a few minutes. The heating was stopped and 0.9 ml of compound **9** were injected using a syringe at an addition rate that maintained reflux (ca 20 min). After the addition was complete, reflux was continued for an additional hour. The reaction mixture was cooled to room temperature. The Grignard thus obtained was transferred via cannula to a solution compound **8** (0.9 g) in 2 ml of THF at 0°C. After 30 min, the reaction mixture was quenched with 1M HCl solution and diluted with ether. The organic phase was separated, dried over Na$_2$SO$_4$, filtered and evaporated to afford 1.5 g of crude material. Purification by flash chromatography on silica gel using 10% ethyl acetate in petroleum ether as the eluent gave 680 mg of pure compound **10**. 3-(1,1-Dimethyl-6-ynyl) resorcinol **(11)** was obtained from compound **10** as described in PCT patent application WO 01/28497.

Scheme 6.

(8)   +   (9)   Mg →

(10)   → →   (11)

(5)   (6)

→

compound F

A mixture of **5** and **6** (1.18 g, 5 mmol), 3-(1,1-Dimethyl-6-ynyl) resorcinol (**11**) (1.18 g, 5 mmol) and p-toluenesulfonic acid (0.95 g, 5 mmol) in chloroform (50 ml) is allowed to react

5   at room temperature for 4 hours. Ether (30 ml) is then added, and the organic phase is washed with saturated sodium bicarbonate, water, then dried over anhydrous sodium sulfate, filtered and evaporated. The residue is allowed to crystallize in acetonitrile to provide 0.5 g of crystals. The mother liquors are chromatographed over silica gel to afford further 0.7 g of pure compound F.
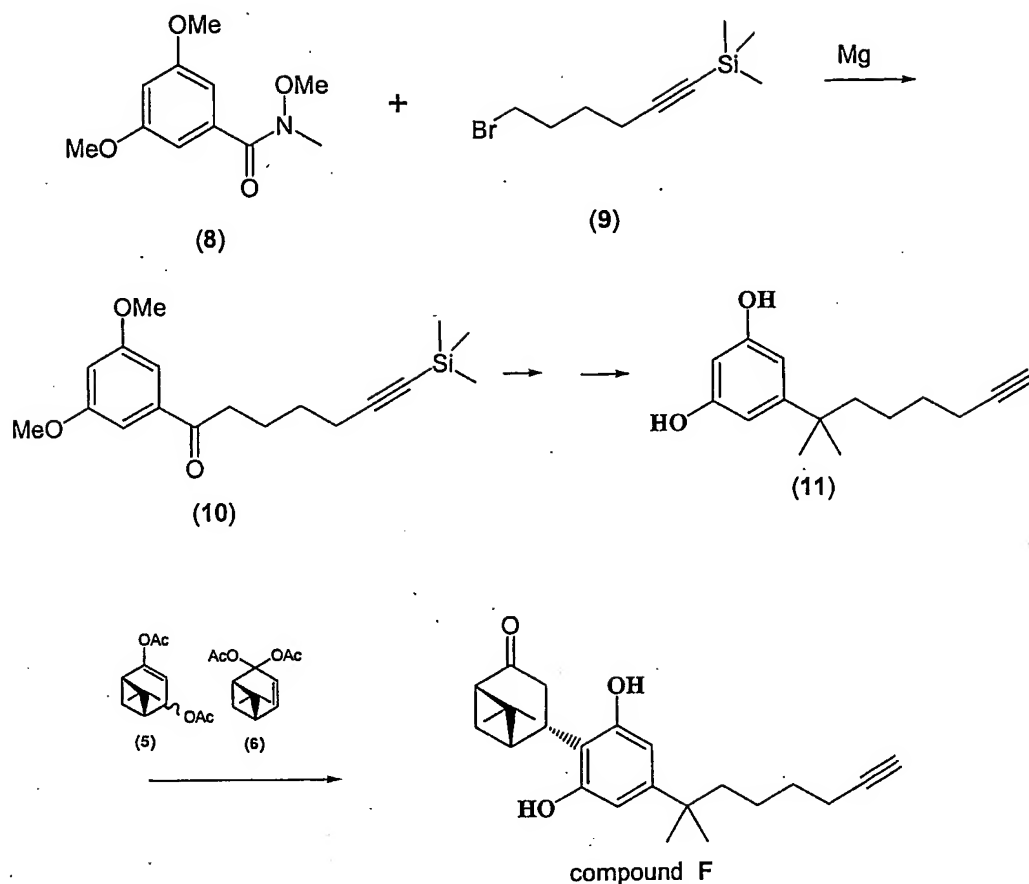
10   **The synthesis of compound G:** (-)-4-[4-(1,1-Dimethyl-3-phenyl-propyl)-2,6-dihydroxy-phenyl] -6,6-dimethyl- bicyclo[3.1.1]heptan-2-one.

The synthesis of compound G is depicted in Scheme 7 when R is ethylbenzene.

35

Compounds **8**, **12** and **13** were prepared as described by Harrington et al. (Harrington P.E. et al., J. Org. Chem. 65: 6576-82, 2000). Compound **14** was prepared as described in the patent application WO 01/28497. Compounds **5** and **6** were prepared as previously described in the synthesis of compound A.

5   Scheme 7.
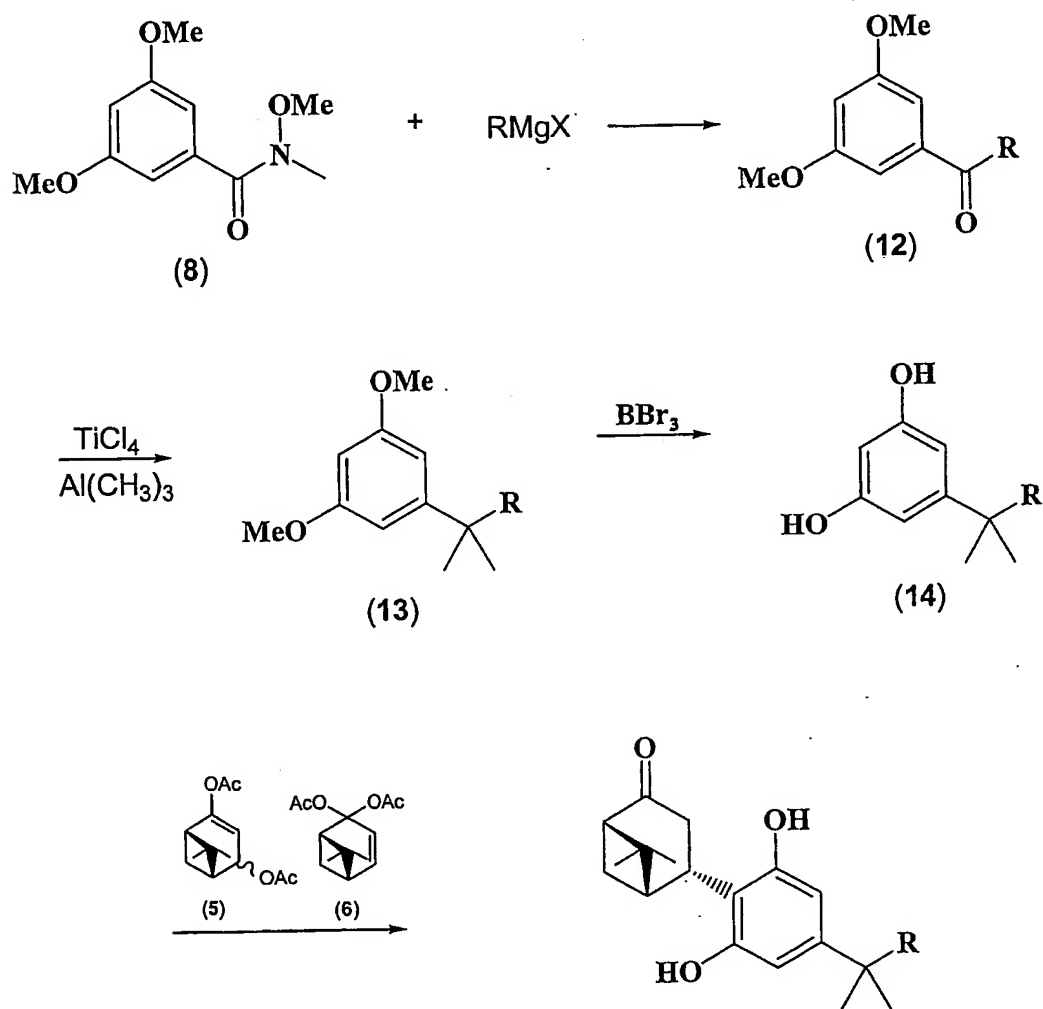
compound **G**: R=Ethylbenzene
compound **H**: R=sec-Butyl
compound **J**: R=p-Chlorobenzene

The synthesis of compound H: (-)-4-[2,6-dihydroxy-4-(1,1,3-trimethyl-butyl)-phenyl]-6,6-dimethyl-bicyclo[3.1.1]heptan-2-one.

The synthesis of compound H is depicted in Scheme 7 when R is sec-butyl. Compounds **5, 6, 8, 12-14** were prepared as described for the synthesis of compounds A and F.

5   **The synthesis of compound J:** (-)-4-{4-[1-(4-chloro-phenyl)-1-methyl-ethyl]-2,6-dihydroxy-phenyl]-6,6-dimethyl-bicyclo[3.1.1]heptan-2-one.

The synthesis of compound J is depicted in Scheme 7 when R is p-chlorobenzene. Compounds **5, 6, 8, 12-14** were prepared as described for the synthesis of compounds A and F.

10

PHYSIOLOGICAL EXAMPLES

Evaluation of the therapeutic effects of the novel bicyclic CB2 ligands is carried out in a series of experimental systems to support the utility of these drugs as immunomodulatory, anti-inflammatory, analgesic, neuroprotective and anti-tumoral agents. These effects are
15   evaluated both *in vitro* and *in vivo*, and are corroborated utilizing the systems described below. Unless otherwise indicated the test compounds are prepared as follows: for in vitro assays the compounds are first dissolved in DMSO and then stepwise diluted in the assay buffer, generally tissue culture medium, down to a final concentration of 0.1% DMSO. For in vivo assays the test compounds are first diluted in cremophor:ethanol (65% and 26.5% w/v
20   respectively) and further diluted 1:20 in physiological buffer, generally saline, to reach the appropriate dose. Thus the vehicle is the original "solvent" diluted in the appropriate buffer.

Example 1
Binding affinity for the CB1 and CB2 receptors.

The CB1 binding assays were performed by testing the ability of the new compounds
25   to displace [^{3}H]CP55940 from the CB1 receptor on membranes derived from hCB1 stably transfected HEK-293 cells (Perkin Elmer/NEN). Membranes were diluted in the assay buffer

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(50 mM Tris-HCl, 2.5 mM EDTA, 5 mM $MgCl_2$, 1 mg/ml BSA, pH=7.4) to 500 µg protein /ml. 50 µl of diluted membranes (25 µg) were incubated with [³H]CP55940 in the presence or absence of the bicyclic test compounds in a total volume of 0.5 ml. Tested compounds were dissolved in DMSO and diluted in the assay buffer to a final concentration of 0.1% solvent.

5   Control samples were added with identical amount of vehicle. Non-specific binding was measured by the addition of 10 µM of WIN 55212-2. Following 1.5 hours incubation at 30°C reactions were filtered through Whatman 934A/H filters (presoaked with 0.1% Polyethylenimine (PEI)).

        The affinities of the novel bicyclic analogs to the CB2 receptor were determined by
10   their ability to displace [³H]WIN 55212-2 from the receptor in membranes derived from hCB2 stably transfected CHO cells (Perkin Elmer/NEN). Membranes were diluted in assay buffer (10 mM HEPES, 1 mM $MgCl_2$, 1mM EDTA, 0.3 mg/ml BSA, pH=7.4) to 500 µg protein/ml. 50 µl of diluted membranes (25 µg) were incubated with 0.8 nM of [³H]WIN 55212-2 in the presence or absence of several concentrations of the bicyclic test compounds
15   in a total volume of 1 ml. Tested compounds were dissolved and diluted as previously described for the hCB1 assay. Non-specific binding was measured by the addition of 10 µM CP 55940. Following 40 minutes incubation at 30°C reactions were filtered as previously described. Filters for all binding assays were counted in a β-counter and log of analog concentration versus % of binding was plotted. IC_{50} values were extrapolated from this plot.

20   The results of the binding assays are shown in Table 1, which depicts the Structure Activity Relationship (SAR) of the preferred compounds, in terms of their ability to displace [³H]WIN 55212-2 or [³H]CP55940 from CB2 or CB1 binding sites, respectively. The values of IC_{50} reported in table 1 were calculated from graphs such as depicted in Figure 1, which shows the binding of selected bicyclic compounds to the cannabinoid receptors. Binding to
25   CB1 is measured by competitive inhibition of [³H]CP55940 in HEK-293 cells stably transfected with the human CB1 receptor gene. Binding to CB2 is measured by competitive inhibition of [³H]WIN55212-2 in CHO cells stably transfected with the human CB2 receptor gene. Both curves (hCB1 ■ and hCB2 ◆), representing % inhibition as a function of compound concentration, are superimposed in this graph. A- Displays the results obtained
30   with compound A. B- Displays the results obtained with compound B. C- Displays the results obtained with compound J. D- Displays the results obtained with compound L.

TABLE 1. SAR and IC_{50} (nM) of novel bicyclic compounds.

COMPOUND	R$_1$	R$_2$ & R$_3$	R$_4$	R$_5$	CB2 IC$_{50}$	CB1 IC$_{50}$	CB2/CB1 affinity ratio
HU-210*					0.322	0.328	1.02
HU-308*		OCH$_3$	DMH	CH$_2$OH	13.3	3600	271
A	O	OH	DMH		0.511	27.6	54
B	O	OCH$_3$	DMH		45	2800	62
C		OH	DMH	OH	3.5	31	9
D	N-OH	OH	DMH		3.4	93	27
E		OH	DMH6	CH$_3$	0.783	26	33
F	O	OH	DMH6		0.344	13	38
G	O	OH	DMPP		6.6	563	85
H	O	OH	TMB		43	1980	46
J	O	OH	CPME		11	659	60
K*		OH	DMP	CH$_3$	1.33	138	104
L	O	OH	DMP		1.8	421	234

* These compounds are included for comparison only.

HU-210 is disclosed in US Patent 5,284,867.

HU-308 and compound K are disclosed in PCT application WO 01/32169.

5   The abbreviations used to define R$_4$ refer to the following substituents:

DMH= 1,1-Dimethyl Heptyl

DMH6= 1,1-Dimethyl Hept-6-ynyl

DMPP= 1,1-Dimethyl-3-phenylpropyl

TMB= 1,1,3-Trimethyl Butyl

10   CPME= 1-(4-chloro-phenyl)-1-methyl-ethyl

DMP= 1,1-Dimethyl Pentyl

Example 2

Anti-inflammatory properties of the bicyclic CB2 ligands in vitro.

Specific aspects of the inflammatory response cascade are mediated by cytokines, such as TNF-α, IFN-γ, IL-2 and IL-1β and by inflammatory mediator such as COX-2 and

5   PGE$_2$. Reducing the levels of these pro-inflammatory agents is very important for the severity of the final inflammatory outcome. These agents are also produced by activated cells of the immune system, and the purpose of this study is to test the impact of the new bicyclic CB2 ligands on secretion of these inflammatory agents from activated macrophages and T cells. The levels of secretion in the various test groups are measured by ELISA assays and the level

10   of inhibition is calculated versus the vehicle treated group.

Quantitation of protein using ELISA.

The technique used to quantify the amount of a given protein in a liquid sample, either tissue culture supernatant or body fluid, is based on Enzyme Linked ImmunoSorbent Assay (ELISA) methodology. Either commercially available or established in house, the assay is

15   based on the capture of the protein of interest by specific antibodies bound to the bottom of an ELISA plate well. Unbound material is washed away, the captured protein is then exposed to a secondary antibody generally labeled with horseradish peroxidase (HRP) or alkaline phosphatase (ALP). Again the unbound material is washed away, the samples are then incubated with the appropriate substrate yielding a colorimetric reaction. The reaction is

20   stopped and reading is performed in a spectrophotometer at the appropriate wavelength. Samples are tested at least in duplicate and the appropriate standard curve, consisting of serial dilutions of the recombinant target protein, is incorporated on each plate. Concentration of the protein in the sample is calculated from the standard curve.

Macrophage activation.

25   RAW 264.7 macrophages, a mouse cell line (ATCC # TIB-71), were grown in Dulbecco's modified Eagle's medium (DMEM) with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, and 10% heat inactivated fetal bovine serum. Cells were grown in tissue culture flasks and seeded at appropriate density into 24 wells tissue culture plates. 0.5 x 10^6 Raw cells in one milliliter were stimulated with 2 μg/ml

30   Lipopolysaccharide E. coli 055:B5 (DIFCO Laboratories). The mouse macrophages were pre-treated for one hour with controls or 10 μM of bicyclic CB2 ligands and later on activated

with LPS. Dexamethasone was used as a positive control at 50 nM. Supernatant was collected 24 hours after activation and the levels of the inflammatory agent under study were determined by ELISA, as previously described. Inhibition was calculated versus vehicle treated cells.

5   Inhibition of IL-1β in activated macrophages.

The results obtained for IL-1β are depicted in figure 2 where the levels of secretion are plotted for each treatment group. From this figure we can see that bicyclic CB2 ligands can be potent inhibitors of IL-1β, compound A inhibits 76% of the secretion, compound D inhibits 67%, compound B inhibits 34% and compound C inhibits 26%. Dexamethasone inhibited 10   97% of IL-1β secretion in the same experiment.

Inhibition of TNF-α in activated macrophages.

The activation of the macrophages is performed as previously described. The levels of TNF-α are measured in an ELISA assay as previously described. Inhibition is calculated versus vehicle treated cells. Treatment with 10 μM of compound A, (-)-4-[4-(1,1-Dimethyl-15   heptyl)-2,6-dihydroxy-phenyl]-6,6-dimethyl- bicyclo[3.1.1]heptan-2-one, reduced TNF-α secretion by 53%.

Inhibition of PGE$_2$ in activated macrophages.

The activation of the macrophages is performed as previously described. The levels of PGE$_2$ are measured in an ELISA assay as previously described. Inhibition is calculated versus 20   vehicle treated cells. Treatment with 10 μM of compound A, (-)-4-[4-(1,1-Dimethyl-heptyl)-2,6-dihydroxy-phenyl]-6,6-dimethyl-bicyclo[3.1.1]heptan-2-one, reduced PGE$_2$ secretion by 49%.

T cells activation.

Jurkat cells (human acute lymphoma T-cell line; ATCC # TIB-152) are grown in 25   RPMI 1640 medium with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES, 1.0 mM sodium pyruvate, and 10% heat inactivated fetal bovine serum. Cells are grown in tissue culture flasks and seeded at appropriate density into 24 wells tissue culture plates. 2 x 10^6 cells in one milliliter are stimulated using 10 ng/ml of PMA (Sigma) and 1 μM A23187 calcium ionophore (Sigma). Cyclosporin A (Sandoz), a 30   known immunosuppressive drug, is used as positive control. The controls and test compounds

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are added at indicated concentrations one hour before stimulation. Supernatant is collected 24 hours after stimulation and the levels of the inflammatory agent under study are measured in an ELISA assay as previously described. Inhibition is calculated versus vehicle treated cells.

Inhibition of IL-2 in activated T cells.

The activation of the T cells is performed as previously described. The levels of IL-2 are measured in an ELISA assay as previously described. Inhibition is calculated versus vehicle treated cells. The results of this experiment are depicted in figure 3 were the levels of IL-2 secretion achieved by vehicle or compounds treated cells are plotted for each concentration. From this figure we can see that bicyclic CB2 ligands can be potent inhibitors of IL-2, compound A has a calculated IC_{50} of 3 μM while compound K has a calculated IC_{50} of 5 μM. Cyclosporin A at a concentration of 10 nM inhibited 98% of IL-2 secretion in the same experiment.

Altogether these experimental results support the conclusion that bicyclic CB2 ligands are potent inhibitors of pro-inflammatory agents secretion from activated cells of the immune system.

Example 3
Effect of bicyclic CB2 ligands on gene expression in cell culture models.

The inhibitory activity displayed by some bicyclic CB2 binding compounds on the secretion of inflammatory agents in activated cells of the immune system may be related to regulation of gene expression.

RNA preparation and real-time RT-PCR.

Total RNA was prepared using SV total RNA isolation system (Promega). The cells or tissues were homogenized in lysis buffer. The lysates were transferred to an RNA isolation column, treated with DNAse, washed and eluted according to kit instructions. RNA concentrations were determined using GeneQuant II (Pharmacia-Amersham). Complementary DNA (cDNA) was synthesized from total RNA using SUPERSCRIPT II reverse transcriptase (Life Technologies). 2 μg of total RNA were combined with an oligo $(dT)_{15}$ primer, 0.5 mM dNTP mix, 8 units of reverse transcriptase and other reaction components up to a final volume of 20 μl, according to the kit instructions. The reaction mixture was incubated at 42°C for 45

min and inactivated at 70°C for 15 minutes. Quantitative real-time RT-PCR includes 1 μl of the cDNA, 300 nM of the appropriate forward and reverse primers (see below) and 7.5 μl of the reaction mix containing buffer, nucleotides, Taq polymerase and SYBR green (SYBR Green master mix, Applied Biosystems), in a total reaction volume of 15 μl. Gene

5   amplification was obtained using the GeneAmp 5700 sequence detection system (Applied Biosystems). Amplification included one stage of 10 minutes at 95°C followed by 40 cycles of a 2-steps loop: 20 seconds at 95°C, and 1 minute at 60°C. During each annealing step, the amount of the amplified product is measured by the fluorescence of the double strand DNA binding dye, SYBR Green. The cycle of threshold (C_T), representing the PCR cycle at which

10   an increase in fluorescence above a baseline signal can be first detected, is determined for each product. A delay of one PCR cycle in the C_T is translated into a two-fold decrease in starting template molecules and vice versa. The changes in the C_T of the specific gene product are normalized to the changes in the C_T of a reference gene cyclophilin or GAPDH. Results are expressed as fold increase of gene expression in the test system above the appropriate

15   control, such as inactivated cell lines or vehicle "treated" animals. In all cases, results are also normalized to either one of the reference house-keeping genes.

Primer sequences used:
Mouse COX-2 forward 5'-TTCCGTTTCTCGTGGTCACTT-3'
Mouse COX-2 reverse 5'- AGCGCTGAGGTTTTCCTGAA-3'

20   Mouse MCP-1 forward 5'-TCACAGTTGCCGGCTGG-3'
Mouse MCP-1 reverse 5'-TCTTTGGGACACCTGCTGCT-3'
Mouse IL-2 forward 5'-GAAACTCCCCAGGATGCTCAC-3'
Mouse IL-2 reverse 5'-GCCGCAGAGGTCCAAGTTC-3'
Mouse IL-10 forward 5'-GCCCTTTGCTATGGTGTCCTT-3'

25   Mouse IL-10 reverse 5'-TCCCTGGTTTCTCTTCCCAA-3'
Mouse cyclophilin forward 5'-TCGCCATTGCCAAGGAGTAG-3'
Mouse cyclophilin reverse 5'-GGTCACCCCATCAGATGGAA-3'
Mouse GAPDH forward 5'-GGTTGTCTCCTGCGACTTCAA-3'
Mouse GAPDH reverse 5'-GTAGGCCATGAGGTCCACCA-3'

30   Human COX-2 forward 5'- TCCTGCCTACTGGAAGCCAA-3'
Human COX-2 reverse 5'- AGCCCTTCACGTTATTGCAGAT-3'
Human IFN-γ forward 5'- CCAACCTAAGCAAGATCCCATG-3'

Human IFN-γ reverse 5'- TTCAAACCGGCAGTAACTGGA-3'

Human IL-2 forward 5'- GGGACTTAATCAGCAATATCAACGT-3'

Human IL-2 reverse 5'- TTCTACAATGGTTGCTGTCTCATCT-3'

Human TNF-α forward 5'- CTGAGGCCAAGCCCTGGTA-3'

5   Human TNF-α reverse 5'- GAGATAGTCGGGCCGATTGAT-3'

Human cyclophilin forward   5'-GCATACGGGTCCTGGCATC-3'

Human cyclophilin reverse 5'-TGCCATCCAACCACTCAGTCT-3'

Human GAPDH forward 5'-ACCCACTCCTCCACCTTTGA-3'

Human GAPDH reverse 5'-CTGTTGCTGTAGCCAAATTCGT-3'

10   <u>Gene expression in LPS activated macrophages.</u>

The mouse macrophages RAW 264.7 are pre-treated for one hour with controls or test compounds and later on activated with LPS, as previously described. RNA samples are extracted from the cells at a predetermined time point after activation and gene expression levels of the inflammatory agent under study are analyzed by real-time RT-PCR as previously

15   described. The results are expressed as fold activation of gene expression over non-activated macrophages, after normalization to cyclophilin expression.

<u>Gene expression in PMA/Ca Ionophore activated T cells.</u>

The human T cells Jurkat are pre-treated for one hour with controls or test compounds and later on activated with PMA and Calcium ionophore, as previously described. RNA

20   samples are extracted from the cells at a predetermined time point after activation and gene expression levels of the inflammatory agent under study are analyzed by real-time RT-PCR as previously described. The results are expressed as fold activation of gene expression over non-activated T cells, after normalization to cyclophilin expression.

Example 4

25   **Gene expression in liver and spleen following ConA injection in mice.**

The transcriptional regulation activity displayed by some bicyclic CB2 binding compounds on inflammation related genes in vitro is reproducible in vivo in models relevant to T cell mediated injury, especially in the liver and the spleen.

<u>The ConA model for T-cell mediated injury.</u>

30   The most common causes of life threatening T-cell mediated liver damage in humans are infections with hepatitis B or C viruses and autoimmune hepatitis. Different animal

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models of autoimmune liver injury have been developed, including acute liver failure in mice induced by intravenous injection of the T-cell stimulatory plant lectin concanavalin A (ConA). ConA has high affinity for the hepatic sinus. Treatment of mice with ConA activates T-cells that accumulate in the liver and release cytokines (such as IL-6, IL-10, TNF-α, INF-γ,

5   IL-2) that regulate liver damage. Pretreatment with the immunosuppressor drugs such as cyclosporin A or FK506 completely prevents liver injury caused by ConA injection, demonstrating the major role of T-cell activation in this model.

Each experimental group contains at least 5 BALB/c inbred female mice (25 g average weight, Harlan, Israel). The negative control group is composed of mice injected with saline

10   instead of ConA. The injection of ConA (Sigma) is done i.v. at the base of the tail at the dose of 10 mg/kg in saline. The treatments are injected i.v. at 1 mg/kg, 30 minutes prior to the ConA injection. Compounds are dissolved in cremophor:ethanol and vehicle only was included as an internal control.

Impact of treatment is monitored at three levels. First, blood samples (200-400 μl) are

15   collected at predetermined time points after ConA injection, using retro-orbital puncture. After short centrifugation (5000 rpm for 2 min) serum is recovered and stored at −80°C until further use for determination of cytokines levels by ELISA and aminotransferase leakage from the liver as a marker for liver injury. In parallel, the level of cytokines is also determined in the organs of interests. For this purpose, the mice are killed by dislocation of the cervical

20   vertebrae, at predetermined time points following ConA injection. The spleen and the liver are removed. Part of the liver is fixed in 4% formaldehyde and the other part was kept at −80°C for protein or RNA extraction. The spleens are weighted and a small part of the spleen is fixed in 4% formaldehyde, while most of the organ is cultured according to the following procedure. Each spleen is squeezed through a cell strainer with the rough end of a 5 ml

25   syringe into 4 ml of RPMI medium. Large tissue fragments are removed by gravity sedimentation and the supernatants are collected. Cells are washed 3 times with 5 ml of erythrocyte lysis buffer (Boehringer), resuspended in 4 ml RPMI medium supplemented with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES, 1.0 mM sodium pyruvate, and 10% heat inactivated fetal bovine serum, and plated in

30   a 6 wells culture dish. Cells are incubated for 24 hours and cytokine levels in the supernatant are determined by ELISA as previously described.

IL-2 gene expression in liver in the ConA model.

The induction of IL-2 expression in the liver of ConA injected mice and the effect of immunosuppressors in such a model have already been reported (Okamoto T. and Kobayashi T., Jpn. J. Pharmacol. 77: 261-3, 1998). The purpose of this study is to check the effect of the

5 bicyclic CB2 binding compounds on the levels of this important pro-inflammatory cytokine.

IL-10 gene expression in liver, and secretion, in the ConA model.

The roles of IL-10 in ConA model of murine liver injury have already been reported and it has been shown that administration of anti-IL-10 antibodies results in aggravated liver injury (Kato et al., Hepatology Research 20: 232-43, 2001). The purpose of this study to

10 check the effect of the bicyclic CB2 binding compounds on the levels of this important anti-inflammatory cytokine.

Example 5

Gene expression in brain tissue following LPS injection.

The transcriptional regulation activity displayed by some bicyclic CB2 binding

15 compounds on inflammation related genes in vitro is reproducible in vivo in models relevant to inflammation. The inflammatory injury is generated by injecting 250 ng LPS in vivo into mice brains i.c.v. PBS is used as control. LPS is dissolved in saline at 50 ng/µl and 5 µl are injected in each ventricule at a rate of 1 µl/min with the help of a syringe pump and a brain infusion cannula. After each injection, the cannula is left *in situ* for one more minute to avoid

20 reflux. The various treatment groups and controls are injected i.p. (0.1 ml/10 g body weight) immediately after i.c.v injection of LPS. Each treatment group is composed of five C57/BL male mice (6-8 weeks old, 25 g average weight, Harlan, Israel). Six hours following LPS injection, the animals are sacrificed by i.p. injection of 100 mg/kg pentobarbitone sodium and their brains are removed and kept at –80°C until next step. RNA is extracted from each whole

25 brain and gene expression levels of inflammatory agents are analyzed by real-time RT-PCR as previously described. The results of this experiment are expressed as fold activation of gene under study in LPS versus PBS injected brains.

This experimental model also allows to monitor the effect of bicyclic CB2 binding compounds on cerebral inflammation by measuring the extent of gliosis. For this purpose the

30 animals are sacrificed 3 days following LPS and treatment injection and their brains are removed. Frozen sections of 20 µm are cut at the level of the hippocampus and stained by standard immunohistochemistry method using antibodies against the F4/80 marker.

Quantitative analysis is performed by counting the F4/80 immunoreactive cells. The differences between the treatment groups are compared using analysis of variance ANOVA followed by post-hoc t-Test. A value of $p<0.05$ is considered to be statistically significant.

Example 6

5   **Gene expression in brain tissue following Middle Cerebral Artery Occlusion.**

The transcriptional regulation activity displayed by some bicyclic CB2 binding compounds on inflammation related genes in vitro is reproducible in vivo in models relevant to neuroprotection.

Transient MCAo in mice

10   This model corresponds to cerebral ischemia as observed in stroke. Mice (C57/BL, male, 25 gr average body weight, Harlan, Israel) are anaesthetized with halothane in 30% oxygen and 70% nitrogen (4% for induction in an anesthesia chamber, and 1-2% in a facemask for maintenance). A midline incision is made in the skin of the neck, and the tissue underneath is bluntly dissected. The right common carotid artery (CCA) and its junction with

15   the external carotid artery (ECA) and internal carotid artery (ICA) are explored by blunt dissection. The branches of the ECA, the occipital and the superior thyroid artery, are then cauterized. The CCA is then transiently closed by positioning around it a 5-0 silk suture material (Assut, Switzerland). Two cm pieces of the nylon suture material are cut and placed in a solution of 1% Poly-L-Lysine and then dried in an oven (60°C) for 60 minutes. The tip of

20   each piece is rounded under a flame. The ECA is permanently occluded with the same type of suture material. A third closure, transient this time, is done in the ICA with 5-0 silk suture material. A small hole is cut in the ECA and the nylon thread is inserted into the ICA while avoiding entrance into the pterygopalatine artery. The thread is inserted 11 mm until a slight resistance is felt. Then a 5-0 silk suture knot secures the thread. One cm of the thread left

25   outside are then cut. The skin wound is closed by 5-0 silk suture material.

Following the operation, the animals are allowed to wake up in the cage. One-hour post insult initiation animals are clinically tested to verify the success of MCA occlusion. The evaluating system was based on works by Belayev et al., (Stroke 27: 1616-23, 1996; Brain Res. 833: 181-90, 1999). It consists of two tests: the postural reflex test and the fore limb-

30   placing test. The postural reflex is evaluated while the animal is suspended by the tail, while the fore limb-placing test is performed while the animal is held by the stomach. Table 1 summarizes the tests and their scoring system.

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Table 1: Neurological evaluation of mice with MCAo.

Item	Normal Score	Deficit
Postural reflex test (hang test) *	0	2
Placing test (performed on each side) #		
Visual placing		
Forward	0	2
Sideways	0	2
Tactile placing		
Dorsal surface of paw	0	2
Lateral surface of paw	0	2
Proprioceptive placing	0	2

* Scores are as follows: 0 no observable deficit, 1 limb flexion during hang test, 2 deficit on lateral push.

Scores are as follows: 0 complete immediate placing, 1 incomplete or delayed placing (>2 seconds), 2 absence of placing.

Only animals with total scores between 8 to12 are included in the study. Ninety minutes after initiation of the insult, the selected animals are resedated using the same method, the neck wound is then re-opened and the nylon thread is pulled out of the ICA. The skin wound is then closed with 5-0 silk suture material. The controls and test compounds are administered 1 minute before the end of the insult. All treatments are delivered i.v. 5 mg/kg. Vehicle is administered 5 ml/kg. Each treatment group comprises 6 to 8 animals. Eighteen hours later, animals are sacrificed by i.p. injection of pentobarbitone sodium 100 mg/kg. Brains are then removed, and total RNA is prepared from the ipsilateral half of the brains. Gene expression levels are analyzed by real-time RT-PCR as previously described. Results are expressed as fold activation over sham operated animals. Gene expression is normalized to house-keeping gene cyclophilin.

MCP-1 gene expression in MCAo brains.

Chemokines are low molecular weight, secreted proteins that chemoattractant and activate specific subpopulations of leukocytes. Monocyte chemoattractant protein-1 (MCP-1)

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is highly specific for monocytes, which are recruited to the site of injury, become activated and secrete inflammatory. Increased MCP-1 RNA levels following MCAo were previously reported (Che et al., Brain Research 902: 171-7, 2001). Immunohistochemistry studies showed that both ischemic neurons (after 12 hours of ischemic insult) and astrocytes (two

5   days after insult) expressed MCP-1. The effect of controls and test compounds on MCP-1 RNA levels in mice brains after 18 hours of MCAo is tested as previously described.

IL-2 gene expression in MCAo brains.

The effect of controls and test compounds on IL-2 RNA levels in mice brains after 18 hours of MCAo is tested as previously described.

10   COX-2 gene expression in MCAo brains.

The effect of controls and test compounds on COX-2 RNA levels in mice brains after 18 hours of MCAo is tested as previously described.

IL-10 gene expression in MCAo brains.

IL-10 is a potent anti-inflammatory cytokine strongly related to the previously

15   described pro-inflammatory genes. Moreover, it has already been reported that IL-10 gene expression levels increase in rat brain following MCAo (Zhai et al., J. Neurol. Sci. 152: 119-24, 1997) and that IL-10 administration reduces rat brain injury following focal stroke (Sperat et al., Neurosci. Lett. 251: 189-92, 1998). We wish to check that this phenomenon can be repeated in the mice model of MCAo and that bicyclic CB2 binding compounds have a

20   further positive impact on the expression of this anti-inflammatory cytokine. The effect of controls and test compounds on IL-10 RNA levels in mice brains after 18 hours of MCAo is tested as previously described.

Example 7

DNA arrays.

25   DNA-array based technologies are widely used in gene regulation research, most commonly to measure differential gene expression, that is comparing the relative level of RNA transcripts in different samples. The purpose of this study is to allow a preliminary screen of the effects of synthetic non-classical cannabinoids on the regulation of a large amount of genes. The technology is based on hundreds (macro-arrays) to thousands (micro-

30   arrays) of sequence-specific DNA fragments spotted on a solid matrix such as glass slides or membranes. RNA samples from the examined tissue or cells are reverse-transcribed into cDNA, labeled and hybridized with the array. The number of labeled transcripts hybridized to

a single spot is turned to a radioactive, fluorescence or chemiluminescence signal and detected by the appropriate instrument. The quantification of the signal on each spot measures the level of expression of the specific gene. We have used membrane-based focused macroarrays each consisting of gene families representing a biological regulatory pathway

5   such as cytokines or chemokine arrays, commercially made by SuperArray Inc. cDNA samples from mice brains after MCAo treated with either vehicle or test compound were labeled with biotin, hybridized to the array-membranes and detected using a chemiluminescence detector, according to SuperArray instructions. The genes that are expressed differentially between treatment and control are subjected to confirmation analysis

10 using real-time quantitative PCR, as previously described.

Example 8

Treatment of inflammation: the ear edema model in the mouse.

The anti-inflammatory activity of the novel bicyclic CB2 ligands was screened in vivo using an ear edema model in mice. This test system utilizes various inflammation inducers,

15 including Croton oil (CO) and Arachidonic acid (AA) and the outcome is assessed by measuring ear tissue swelling. Nonsteroidal anti-inflammatory drugs have been shown to reduce swelling in this model (Young, J.M. et al., J. Invest. Dermatol. 82: 367-71, 1984). The ability of the test compounds to prevent or diminish the inflammatory response to these stimulants is indicative of their systemic anti-inflammatory capability.

20 Compound A, (-)-4-[4-(1,1-Dimethyl-heptyl)-2,6-dihydroxy-phenyl]-6,6-dimethyl bicyclo[3.1.1]heptan-2-one, was dissolved in cremophor:ethanol (respectively 65% and 26.5% w/v) and injected i.p. in adult male ICR mice (30 g average body weight, Harlan, Israel) after dilution with sterile 0.9% sodium chloride to desired final concentrations according to required doses. Various doses of compounds were checked ranging from 0 to 30

25 mg/kg. Each treatment group was composed of 8-10 animals while the vehicle treated group was composed of 16 animals. Inflammation was immediately induced by applying 20 µl of 50% CO in acetone to the outer surface of one ear, the contralateral ear was exposed to acetone only and served as control. Ear thickness was determined (in 0.01 mm units) 3 hours after CO application using a dial thickness gauge (Mitutoyo, Japan). Finally the ears were

30 trimmed, an ear punch of 6 mm diameter was removed and its weight was measured. The ear edema is expressed as the ratio of ear punch weight of the CO treated ear versus the contralateral Acetone treated ear. Results are calculated as % inhibition as compared to

cremophor:ethanol vehicle treated animals. From the analysis of the dose response performed in this study we see that compound A has an ED_{50} of 30 mg/kg or 81 µmole/kg when injected intraperitoneally. These results show that bicyclic CB2 ligands can function as systemic anti-inflammatory compounds.

5   **Example 9**

Experimental autoimmune diseases: CIA, EAE and DTH.

Autoimmune diseases are associated with elevated levels of inflammatory cytokines. The rodent models most commonly studied are experimental allergic encephalomyelitis (EAE), a model for multiple sclerosis in the human, experimental autoimmune arthritis, a model for
10 rheumatoid arthritis in the human and delayed type hypersensitivity (DTH), a model for allergic reactions in the human. EAE is an autoimmune neurological disease elicited by sensitization of the animals to myelin basic protein from the central nervous system, which is also known as basic encephalitogenic protein. Experimental autoimmune arthritis is induced in animals by immunization with collagen in complete Freund's adjuvant: the model is therefore named
15 collagen induced arthritis (CIA). Delayed type hypersensitivity is induced by the application of dinitrofluorobenzene according to a strict time-schedule, therefore the model generated correspond to allergic contact dermatitis in the human. The purpose of the present study is to test the ability of our compounds to prevent or attenuate the clinical signs of these three autoimmune disease models.

20 <u>Collagen Induced Arthritis.</u>

Adult DBA/1 male mice (20 g average body weight, Harlan, Israel), at least eight per treatment group are used in this study. Bovine collagen type 2 is dissolved in 0.05 M acetic acid at a concentration of 2 mg/ml by stirring ON at 4°C. The collagen solution is further emulsified in an equal volume of Complete Freund's Adjuvant (CFA). Each animal is
25 administered with 100 µg collagen type 2 in 0.1 ml CFA emulsion. The collagen is administered s.c. at the base of the tail. Twenty-one day after priming, the mice receive an intradermal booster injection of 100 µg collagen in Incomplete Freund's adjuvant.

The volume of each hind paw is measured using a plethysmometer (Hugo Basill, Italy), and the thickness using a dial, constant pressure gauge, (Mitutoyo, Japan).
30 Measurements are performed before collagen administration and every second day throughout the designated follow-up period. All treatments are administered intraperitoneally. At the end of the treatment period the animals are sacrificed with pentobarbital 100 mg/kg i.p.

The differences between the severity of the paw swelling among various treatment groups are compared using analysis of variance ANOVA followed by post-hoc t-Test. A value of $p < 0.05$ is considered to be statistically significant.

Experimental Autoimmune Encephalomyelitis.

5   Various animal models of autoimmune encephalomyelitis are known in the art, depending on the method of induction, the strain of the animal and the antigen employed to induce the disease. The impact of bicyclic CB2 ligands was tested in EAE using Lewis rats in which the onset of disease is observed by the appearance of clinical symptoms about 10 days after induction. The disease progress and the clinical score increase and peak around day 15 and

10   spontaneous recovery is observed around day 18 after induction of the disease. The animals (at least 9 per test group at initiation of study, except for the untreated control group that comprised only 5 rats) were maintained on a 12 hours light/12 hours dark regimen, at a constant temperature of 22°C, with food and water ad libitum. EAE was induced in these animals by immunization with s.c. injection to the hind paws of 25 µg of purified guinea pig myelin basic

15   protein (MBP, Sigma) emulsified in 0.1 ml of Complete Freund's Adjuvant (Difco).

Animals that exhibited symptom of the disease, which could be clinically scored between 0.5 and 1, were treated with test compounds or vehicle control, administered intravenously in a volume of 5 ml/kg, for three consecutive days starting from the onset of the disease (~ at day 10 following disease induction). Methylprednisolone was used as positive

20   control and it was administered daily for 5 consecutive days i.v. at 30 mg/kg starting from day of disease induction by MBP injection. The results are recorded as clinical score; score of 0 indicates a normal animal with no clinical signs, 0.5 indicates a loss of tonicity in the tail's distal part, 1 indicates whole tail paralysis, 2 indicates paraplegia, 3 indicates quadriplegia, 4 indicates complete body paralysis and moribund state and 5 indicates death. The clinical score

25   of the animals is recorded for 11 days following onset of disease and the area under the curve (AUC) is calculated over this period of time. The differences between the severity of the clinical outcomes among various treatment groups was analyzed by analysis of variance (ANOVA) followed by Fisher's LSD test. A value of $p < 0.05$ is considered to be statistically significant.

30   Results are displayed in figure 4 as the % of reduction in the average AUC for each treatment group. Compound A yielded a reduction in the AUC of the clinical score in a dose related manner, with a significant reduction of 35% at the dose of 1 mg/kg. Compound K

yielded a reduction of 30% in the AUC of the clinical score at the dose of 2.5 mg/kg. These two optimal results obtained with 1 mg/kg of compound A and 2.5 mg/kg of compound K, as well as other treatment groups noted by a # in figure 4, are statistically better (p<0.05) than the results obtained with untreated and vehicle treated animals. In this experimental setup, the

5   positive control methylprednisolone (MPred) yielded 34% reduction when administered 5 times before the disease onset at the dose of 30 mg/kg. Benzyl alcohol served as MPred's vehicle and by itself increased the AUC by 18%, data not shown in figure. These experimental results suggest that bicyclic CB2 ligands are effective treatments in model relevant to human multiple sclerosis.

10 <u>Delayed Type Hypersensitivity in mice model.</u>

· Adult female BALB/c mice (20 g average body weight, Harlan, Israel) were sensitized on day 0 and day 1 by application of 30 µl of 0.15% Dinitrofluorobenzene (DNFB) diluted in acetone on the shaved skin of the abdomen. On day 6 the animals were challenged by application of 10 µl of DNFB in acetone on one ear. The contralateral ear was not challenged

15 but received the application of 10 µl acetone. Test compounds were administered at increasing doses from 0 to 15 mg/kg i.p. twice, the first injection was immediately after DNFB challenge (on day 6) and the second injection was 16 hours post challenge (on day 7). Each treatment group comprised at least 7 animals. Dexamethasone (DXM) was used as positive control. Ear thickness was determined (in 0.01 mm units) 24 hours after challenge

20 (and 6 hours after second treatment on day 7) using a dial thickness gauge (Mitutoyo, Japan).

Results are analyzed as ear thickness of DNFB treated over DNFB untreated contralateral ear. The impact of the test compound was further assessed by comparing its mean impact on the animals of the treatment group to the response generated by the appropriate vehicle only. Results are displayed in figure 5 where % of reduction in ear

25 thickness is plotted against the treatment dose. Generally speaking the pattern obtained is that of a curve reaching a plateau of activity. For the positive control we can see that the maximal inhibition is around 80% while for HU-308 the maximal inhibition is in the range of 60%. The calculated IC_{50} are 3.2 mg/kg for dexamethasone and 4.8 mg/kg for HU-308. Compounds A and K do not reach 50% inhibition and their maximal reduction is in the range of 30-40%.

30 These experimental results suggest that bicyclic CB2 ligands are effective treatments in model relevant to human allergies and immune responses.

Example 10

Treatment of neurodegenerative disorders: the MPTP model.

Parkinson's disease (PD) is a neurodegenerative disorder characterized by tremor, slowness of movements, stiffness and poor balance. Most, if not all, of these disabilities are due to a profound reduction in striatal dopamine content caused by loss of dopaminergic neurons in the Substantia Nigra pars compacta (SNpc) and of their projecting nerve fibers in the striatum. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a well known neurotoxin that can cause depletion of dopamine content in the striatum and a reduction in the number of nigrostriatal dopaminergic neurons in several species including humans (Turski L. et al., Nature 349: 573, 1991). The aim of the present study was to examine the effect of bicyclic CB2 ligands on the progression of MPTP-induced dopaminergic toxicity.

Animal treatment and procedure.

The mice (C57/BL male mice, average weight 30 g, Harlan, Israel) are administered i.p. with 4 injections of MPTP (Sigma, USA) (20 mg/kg, 5 ml/kg) in saline (Teva Medical Israel) at 2 hours interval on day 1. The treatment groups were: (a) Saline, untreated, (b) MPTP, untreated, (c) Vehicle (1:20 of cremophor:ethanol 65%:26.5% w/v), 5ml/kg i.p. once just before the first MPTP administration, and (d) HU-308, 20mg/kg i.p. once just before the first MPTP administration. Seven days following the MPTP treatment the animals are sacrificed (by i.p. administration of pentobarbitone sodium CTS Israel 100 mg/kg) and their brains are removed for tyrosine hydroxylase (TH) detection using immunohistochemistry technique.

Immunohistochemistry.

Brains are fixed by cardiac perfusion with 4% Paraformaldehyde followed by immersion of the brain in the same fixative for at least 72 hours. Then brains are washed with PBS and transferred to 30% sucrose in PBS until they sink. After the brains sank in the sucrose they are frozen using the cryostat special fast freezing (-60°C). The brains are then cryosectioned (20 µm) at the level of the Substantia Nigra (SN). Immunohistochemistry staining is performed using rabbit anti-tyrosine hydroxylase (1:100, Calbiochem). The slides are stained using diaminobenzydine (DAB) detection kit of automated immunostaining system (Ventana). Quantitative analysis is performed by counting of immunoreactive (IR) cells at the widest dimension of the SNpc lateral to the roots of the third cranial nerve

separating medial and lateral SN at the level of interpreduncular nucleus. The amount of the labeling at the striatum level is evaluated using computerized image analysis system.

All data are expressed as mean±SD. Data were analyzed using analysis of variance (ANOVA) followed by post-hoc Fisher test. A value of $p < 0.05$ is considered to be statistically

5   significant.

TH immunoreactivity at the level of the SNpc.

Figure 6 shows the effect of HU-308 in the MPTP model for Parkinson's disease. The number of TH-IR cells/mm^2 at the level of the SNpc following MPTP injection and treatment is plotted for each treatment group. Black column- saline injected untreated group. Black

10   dotted column- MPTP injected untreated group. Hatched column- MPTP injected treated with compound vehicle. White column- MPTP injected group treated with HU-308. The signs above the columns refer to the statistical analysis: # $p < 0.05$ compared to saline; * $p < 0.05$ compared to vehicle. Following MPTP injection the number of TH-IR cells decreased by 65% comparing to the saline treated animals (58±10 saline group vs. 20±3 MPTP group).

15   Calculating the TH-IR results from the treated groups relative to the MPTP treated group revealed that HU-308 rescued about 43% of SN dopaminergic cells from MPTP toxicity. The vehicle had by itself no rescue effect on TH-IR cells. These results show that bicyclic CB2 ligands, are effective in models for chronic neurodegenerative diseases such as Parkinson's disease.

20   **Example 11**

Treatment of chronic neuropathic pain: attenuation of mechanical allodynia.

The aim of this study was to assess the potential analgesic effects of the novel bicyclic CB2 binding compounds in an animal model of neuropathic pain. A peripheral monopathy was induced in the right hind limb of rats following a chronic constriction of the sciatic nerve

25   (Bennet, G.J. & Xie, Y-K., Pain 33: 87-107, 1988). The development of mechanical allodyna was monitored using an established behavioral test (Von Frey filaments).

Pre-surgery baseline values are ascertained as the mean of 2 pre-surgery values. Once the baseline values had been established, the animals were surgically prepared by constricting the right sciatic nerve with 4 chromic cat gut loose ligatures. On day 11 post-operation, the

30   animals that have developed mechanical allodyna were arbitrarily allocated to the various treatment groups based on the pre-surgery values.

The design is randomized, performed in a masked fashion as to whether drug or vehicle is being given. The animals, male Sprague-Dawley rats (average body weight 240-290 g), are allowed to acclimatize to the behavioral testing equipment prior to testing. On the testing day, the animals, at least six per treatment group, are given i.p. a single dose of one of the test compounds in a volume of 2.5 ml/kg. Fifteen minutes later, a series of Von Frey filaments (pre-calibrated prior to testing) were applied to the plantar surface of the hind paw, from below. The filaments are applied in ascending order starting with the weakest force and the withdrawal threshold for both the ipsilateral and contralateral hind paws is evaluated. Each filament is indented on the mid-plantar surface of the foot to the point where it just starts to bend; this is repeated approximately 8-10 times per filament at a frequency of approximately 1 Hz. The withdrawal threshold is defined as being the lowest force of two or more consecutive Von Frey's filaments to elicit a reflex withdrawal response (i.e. a brief paw flick) and is measured in grams.

Figure 7 shows the effect of compound A in the Chronic Constriction Nerve Injury model for Neuropathic pain. The results are expressed as % increase in the threshold of response to Von Frey's filaments in the test compound treated group versus the vehicle treated animals and per definition the vehicle treated group yields a null baseline value. The black column represents the morphine treated animals (4 mg/kg), the gray and the dotted column bars represent two doses of compound A (0.5 and 1 mg/kg respectively). From this study it appears that 15 minutes following treatment, animals treated with Morphine at a dose of 4 mg/kg have a 91% higher pain threshold in their ipsilateral hind paw than those of the vehicle treated group. The groups treated with 0.5 mg/kg and 1 mg/kg of compound A display respectively 71% and 64% improvement in their pain threshold while in a separate experiment the animals treated with 5 mg/kg of HU-308 show 117% improvement (data not shown). These results teach that bicyclic CB2 ligands can alleviate or treat chronic neuropathic pain.

Example 12
Treatment of acute peripheral pain: the tail flick model.

The aim of this study was to assess the potential analgesic effects of the novel bicyclic CB2 binding compounds in an animal model of acute pain. In this model the nociceptive

stimulus is thermal and the latency time till the animal flick his tail is monitored (Le Bars D., Gozariu M. & Cadden S.W., Pharmacol. Rev. 53: 597-652, 2001).

ICR male mice (20-30 g average body weight, Harlan, Israel) were injected i.p. at the volume dose of 5 ml/kg. Each treatment group contained at least 5 animals. Morphine HCl
5   was used as positive control at the final dose of 5 mg/kg. Its vehicle, saline, was also included as control. The test compounds were dissolved in cremophor:ethanol and diluted 1:10 in saline prior to injection, this second type of vehicle was also included as negative control. The final dose injected varied from 0.1 to 10 mg/kg. At predetermined time points after treatment injection, the animals were placed in the tail flick system (Socrel, model DS 20). Animals
10 were gently held while their tails were located above the photoelectric cell. The tails were then illuminated (21V) at 2 cm from the distal tip and the latency time, measured in seconds, was recorded in duplicates. At the end of the study, the animals were euthanized by i.p. injection of 100 mg/kg sodium pentobarbitone.

The differences between the latency times among various treatment groups was
15 analyzed by analysis of variance (ANOVA) followed by post-hoc Tukey's test. A value of $p < 0.05$ is considered to be statistically significant.

Figure 8 shows the effect of various doses of compound A (dotted columns) and compound K (hatched columns) in the Tail Flick model for acute pain. When the measurements were performed 30 minutes after injections (panel A), the latency times for the
20 two control groups were similar, 2.65 sec for saline and 2.89 sec for test compounds' vehicle. The positive control morphine increased the latency time to 7.5 sec at 5 mg/kg ($p < 0.05$ as compared to saline). Test compound A significantly ($p < 0.05$ as compared to vehicle) increased the latency time at all doses tested from 2 to 10 mg/kg, with a maximal latency of 7.1 sec at maximal tested dose. Test compound K significantly increased the latency time to
25 4.73 sec at 2 mg/kg, but shows no further improvement at higher doses. When the measurements are performed 90 minutes after injections (panel B), the effect of Morphine is significantly reduced and its latency time is now of only 3.9 sec whereas the effects of compounds A and K remain relatively stable. At the optimal dose tested, 10 mg/kg, compound A still yields a latency of 7.3 sec 90 minutes after injection, while compound K
30 maximum latency at 4 mg/kg is identical to Morphine performance of 3.9 sec. Significant analgesia was still evident at dosages of 8 and 10 mg/kg of compound A even 150 minutes

after injection. These results teach that bicyclic CB2 ligands can alleviate or treat acute peripheral pain.

Example 13

Formalin test for peripheral pain.

5   Pain mediated by the peripheral nervous system, is tested in the 'formalin test' for cutaneous (peripheral) pain   (Tjolson, A. et al., Pain 51: 5-17, 1992).   First the bicyclic compound (or its vehicle) is injected i.p. In experiments which involve an antagonist, either to CB1 or CB2, the latter is administered i.p. 15 min before the bicyclic test compounds. Formalin is injected s.c. in the hind paw of a mouse 90 min after the test compound.

10   Immediately after formalin administration pain is assessed (every 5 min for 1 hr) by the number of times the animal licks the formalin-injected paw.

Example 14

Diabetes type I: the NOD mice model.

The purpose of the present study is to establish a model in non-obese diabetic (NOD)

15   mice to test the protective activity of bicyclic CB2 binding compounds in an experimental setup relevant to human insulin-dependent diabetes mellitus.

NOD/lt female mice (70-80 days old at study onset, Harlan, Israel) are weighted at day 1. Their baseline glucose level is established using a drop of blood obtained by sectioning ·the tip of the tail and a glucometer with the appropriate glucosticks (Elite, Bayer). Mice are

20   then injected i.p. with cyclophosphamide (Sigma) diluted in saline at a dose of 300 mg/kg. The appearance of glucose in the urine of the animals is monitored every two days using a urine multistick (Bayer). When this test indicates that the animals reach glucourea, then the level of glucose in the blood is reassessed during two consecutive days after overnight starvation. Animals are defined as diabetic if their glucose blood levels are above 300 mg/dl.

25   Three days following the diagnostic of diabetes, the animals are sacrificed by i.p. injection of 100 mg/kg pentobarbitone. Their spleen and pancreas are removed for further study including FACS analysis of the T cells subpopulations in the spleen and histo- and immuno-pathological evaluation of the pancreas.

The histopathological evaluation is performed on ten Langerhans islands for each

30   animal and the scoring is according to the following method (Sempe P. et al., Eur. J. Immunol. 21: 1163-9, 1991). The severity of the damage is scored according to the level of

mononuclear infiltrate: 0- no infiltration, 1- periductular infiltrate, 2- peri-islet infiltrate, 3- intra-islet infiltrate, 4- intra-islet infiltrate associated with β-cell destruction. The mean score for the pancreas of each animal is calculated by dividing the total score by the number of islets examined.

5 **Example 15**

Renal ischemia.

The purpose of the present study is to test the nephro-protective activity of bicyclic CB2 binding compounds in an acute renal ischemia model in rats.

Male Sprague Dawley rats (250 gr average body weight, Harlan, Israel) are
10 anesthetized with a combination of xylazine and pentobarbitone 8 and 35 mg/kg i.p. respectively. Then a 45-minutes ischemia is induced bilaterally on both kidneys. The sedated animals are positioned on their backs. The abdomen skin is shaved and cleaned with 70% ethanol. A midline skin incision is performed (2-3 cm long) and the abdomen is opened through an incision in the *linea Alba*. The kidneys are explored after gentle removal of the
15 intestines to the opposite direction. While this is done, the intestines are covered with wet (warm saline 37°C) sterile sponges. The renal arteries are isolated by blunt dissection from the surrounding fat, and occluded together with the renal veins in the kidney hilus by arterial micro clips (FST Canada). Kidneys that become pale immediately after artery occlusion are considered ischemic. Only animals showing that both kidneys are ischemic are included in the
20 study. During the ischemic insult the intestines are returned into the abdominal cavity. The wound is covered with wet sponges (they were kept wet by rinsing warm saline). In addition, rectal temperature is monitored to remain between 37°C-38°C. Rectal temperature is measured using a thermistor (YSI USA model 400) and a measuring unit (Cole Parmer model 8402-00).

Forty-five minutes after the ischemia initiation, the artery clips are removed.
25 Reperfusion is verified by the return of the pink color of the kidney. The wound is then closed with 3-0 silk suture material (Assut, Switzerland) in two layers (abdomen wall and skin). At 1, 3 and 7 days post ischemic insult animals are lightly anesthetized in an anesthesia chamber with ether and blood samples are collected after an infra orbital sinus puncture. Blood is collected into eppendorf tubes, and centrifuged (4000 rpm for 5 minutes). Serum is then
30 separated and kept at -20°C prior to evaluation of blood levels of creatinine and blood urea nitrogen (BUN). At the end of the study, animals are euthanized with pentobarbitone sodium

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100 mg/kg i.p. Kidneys are removed, weighted and kept in 4% formaldehyde solution for possible further usage.

Treatments are administered i.v. into the femoral vein at 5 ml/kg to 10 animals per group, immediately after the end of the ischemic insult. Results are compared to ischemic 5 (vehicle treated) and sham (the same procedure, without renal artery occlusion).

The blood levels of BUN and creatinine are compared using ANOVA followed by Duncan's post-hoc test.

Example 16

Adenylylcyclase assay.

10   Cannabinoids and derivatives bind to G-protein-coupled CB1 and CB2 receptors and exert their activity via the inhibition of adenylylcyclase activity. An adenylylcyclase assay forms a basis for the functional analysis of the compounds by determining their capacity inhibit or promote forskolin-activated cAMP production. The assay is carried out according to Chin et al. (Chin, C.N. et al., J. Neurochem. 70: 366-73, 1998). Briefly, HEK-293 human 15 kidney cells stably transfected with either the human CB1 or CB2 receptor are grown in DMEM supplemented with 10% fetal calf serum, 1% Penicillin-Streptomycin and 2 mM L-glutamine. Cells are seeded in 24 wells plate at 1×10^5 cells/well and incubated for 48 hours. Medium is then removed and the adherent cells are washed with PBS. Hundred µl of serum free medium supplemented with 0.2 mM Ro 20-1724, 0.25% BSA, and 20 mM HEPES is 20 then added to each well. The cells are then activated with 1 µM forskolin in presence of the bicyclic test compounds. The activated cells are then incubated at 37°C for 20 minutes and the reaction terminated with 1.2 M HCl to a final concentration of 0.1 M. Cells are lyzed by freeze and thaw and the lysate is neutralized with 2 M HEPES, pH 7.5. cAMP is measured in 50 µl aliquots using the [^{3}H]-cAMP assay system (Amersham).

25   In addition, mammalian cells, stably expressing exogenous human CB1 or CB2 receptors and a luciferase reporter gene linked to the cyclic-AMP response element (CRE) are activated with different stimuli, such as forskolin or calcium ionophore. Following activation, the cells are extracted and the activity of the reporter gene is measured in luminescence units by the luciferase assay. Elevation in cyclic-AMP is reflected by an increase in luciferase 30 activity.

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Example 17

Psychomimetic effects of the compounds.

Female ICR mice (2 months old, Harlan, Israel) are used for a series of tests for psychotropic effects, specifically blood pressure, heart rate, ECG, locomotor activity and

5   rectal temperature. The test compounds, and known and specific CB1 and CB2 antagonists when appropriate, are dissolved in vehicle and injected i.p. at a dose of 20 mg/kg in volumes not exceeding 0.1 ml/10g in mice.

A series of four consecutive observations are performed on each mouse following a standard procedure previously described (Martin, B.R. et al., Pharmacol. Biochem. Behavior,

10   40: 471-8, 1991). Briefly, 30 minutes after i.p. injection, mice are tested sequentially in four assays: motor activity (ambulation and rearing) in an open field (60 x 50 cm) for 3 min; immobility ("catalepsy") on a ring of 5.5 cm diameter for 4 min; body temperature with a telethermometer (Yellow Springs Instruments Co.); analgesia on a hot plate maintained at 55°C was measured as the latency (in seconds) until the first hind paw lick or jump from the

15   plate (the latter response rarely observed) with a maximum of 45 s.

Differences between vehicle and treatments are compared by one-way ANOVA, followed by post-hoc Newman-Keuls tests (Prism software from Graphpad, San Diego). A value of $p < 0.05$ is considered to be statistically significant.

Example 18

20   **Effect of the compounds on blood pressure.**

Systemic blood pressure is measured in male Sprague Dawley rats (270-350 g, Harlan, Israel). The test compounds are dissolved in vehicle and injected i.v. at a dose ranging from 0.5 to 10 mg/kg in volumes of 0.1 ml/100g body weight. A chronic cannula (P 50, Clay Adams) is implanted into the femoral artery under pentobarbital anesthesia (60 mg/kg). The

25   vein is cannulated for drug administration. The arterial cannula is attached to a pressure transducer (Db23, Statham City). The transducer is connected to a data acquisition system (Biopac, USA). Recordings are taken for 30-60 min both before treatment and following i.v. bolus injections of the test compounds.

Differences between vehicle and treatments are compared by one-way ANOVA, followed by post-hoc Newman-Keuls tests (Prism software from Graphpad, San Diego). A value of $p < 0.05$ is considered to be statistically significant.

Example 19

5   **The Langendorff perfusion model for measuring cardioprotection.**

Endogenous cannabinoids were recently shown to be involved in the cardioprotective effect of LPS against myocardial ischemia (Lagneux, C. & Lamontagne, D., Br. J. Pharmacol. 132: 793-6, 2001). The cardioprotective effect of the novel bicyclic compounds is tested in the Langendorff model of the isolated perfused rat heart. Male Sprague-Dawley rats weighing
10   280 ± 20 g are used for perfusion experiments in compliance with the NIH *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 85-23, revised 1996). The animals are injected intraperitoneally with sodium heparin (500 U) and anesthetized with pentobarbital (30 mg/animal). Hearts are immediately removed and placed in heparinized ice-cold saline solution. The aorta is cannulated to a Langendorff perfusion apparatus and the pulmonary
15   artery cut open to provide free drainage of effluent. Retrograde aortic perfusion is maintained with modified Krebs-Henseleit (KH) solution. The KH is aerated with a mixture of 95% oxygen and 5% carbon dioxide. Aortic perfusion is maintained at 37°C, at a constant pressure of 90 cm H_2O.

Short Term Ischemia at normothermia.
20   Hearts undergo 20 min of KH perfusion, 25 min of no-flow global ischemia (at 37°C), and 45 min of KH reperfusion. This has been shown to reduce the work index (LVDPxHR) recovery to ~40%, a potential for improvement by drugs. Different concentrations of the compounds are added during pre-ischemic perfusion, reperfusion or both.

Hemodynamic Measurements.
25   A latex balloon-tipped catheter is inserted through a small cut in the left atrium and advanced through the mitral valve into the left ventricle. The balloon is connected, through a pressure transducer, to a recording system (Hewlett Packard 7758B, USA). The balloon is inflated and equilibrated to give an end-diastolic pressure of 0 mm Hg. Left ventricular systolic and diastolic pressures and time derivatives of pressure are measured during
30   contraction (+dP/dt) and relaxation (-dP/dt). Left ventricular developed pressure (LVDP) is calculated from the difference between the systolic and diastolic pressures. The work index of

the heart (LVDPxHR) is calculated from the product of LVDP and heart rate (HR). Coronary flow (CF) is measured by collecting the effluent drained through the pulmonary artery in the pre-ischemic period and during reperfusion. Hearts are excluded from the study if arrhythmias develop, thrombus forms, or LVDP and heart rate after the first 20 min of perfusion is less

5   than 60 mm Hg, and 210 beat/min respectively.

Results are expressed as Mean±SEM. Statistical differences between groups of hearts are calculated using the ANOVA and Mann-Whitney rank tests A value of $p<0.05$ is considered to be statistically significant.

Example 20

10   **Effect of compounds tumor cell lines and tumors.**

In vitro.

Cells from several tumor-derived cell lines are tested for their proliferation capacity in presence of our test compounds. Tumor cell lines are obtained from ATCC and grown according to supplier recommendation. Cells are seeded in a 24 well plate (10^5 cells/ml/well)

15   and grown overnight. The cells are incubated with the test compounds (1–100 μM) or vehicle (0.1% DMSO final concentration). Cell viability is determined 24 hours later using standard crystal violet staining. The culture medium is removed from the wells and the cells are fixed by adding 1 ml/well of 2% formaldehyde in PBS for 10 minutes. Following fixation the cells are washed three times with PBS and 250 μl of 0.5% (w/v) crystal violet is added to each well

20   and the plates are incubated for 30 minutes at room temperature with gentle agitation. The stained cells are then washed three times with double distilled water and the color is extracted by adding to each well 250 μl of 10% acetic acid. The plates are agitated for 15 minutes at room temperature and 100 μl are transferred in duplicate to a 96 well plate for reading. Optical density (OD) of the cells is measured at 620 nm in an ELISA reader and results are

25   expressed as % viable cells. Absorbance of untreated cells is recorded as 100%. The IC_{50} (dose inhibiting cell growth by 50%) is determined.

Moreover, the cells are stained for activated caspase 3 to determine whether they died through an apoptotic mechanism. The medium from the wells is discarded and cells are fixed by adding 1 ml of 4% formaldehyde in PBS, for 10 min. Cells are washed twice with PBS-

30   0.1% Tween20 (PBS-T) and permeabilized with cold methanol for 20 min. The cells are washed twice with PBS-T and incubated with 1 ml blocking solution (3% BSA, PBS-T) for

30 min. The primary antibody (rabbit anti- cleaved caspase 3 (asp175) Cell Signaling Technology, diluted 1:50 with blocking solution) is added and the cells incubated for 60 min. at 37°C. The cells are washed twice with PBS-T. The secondary antibody (HRP conjugated anti-rabbit IgG diluted 1:200 with blocking solution) is added to the wells and incubated for

5   60 min. at RT. Cells are washed twice with PBS-T and incubated for 10 min with a fluorescein tyramide reagent (NEN, diluted 1:50 with amplification diluent). Cells are washed twice with PBS-T and the signal visualized by fluorescence or confocal microscope. Beside monitoring activated caspase-3, the expression of apoptosis-related genes in cells treated with dexanabinol and its analogs is compared to that in untreated cells. The procedure

10   for real-time RT-PCR is as previously described. For each gene, a pair of specific PCR primers is designed and the reaction is done according to the ABI protocols. The quantification of level of expression of each gene is normalized to a housekeeping gene and compared to RNA samples from non-treated cells.

<u>In vivo.</u>

15   Once we have selected the tumor cell lines whose proliferation is inhibited by the bicyclic CB2 binding test compound in vitro, we test the efficiency in vivo. Cells are grown according to supplier recommendation. Predetermined amounts (1×10^6 cells in constant volume of 0.12 ml/animal) are injected s.c. above the right femoral joint in nude CD-1 male mice (average weight 20-25 gr, Harlan, Israel). Each treatment group is composed of at least 7

20   animals. Each animal is clinically monitored daily. The growth of the tumor is also monitored during the daily visits but actual measurements are recorded once a week. When tumors reach the appropriate size, animals are treated with either vehicle, 5 ml/kg/day, or with our test compounds, in the range of 2.5 to 10 mg/kg/day.

Example 21

25   **Effect of the compounds in the model for inflammatory bowel disease.**

The purpose of this study is to evaluate the activity of test compounds in a masked study of acetic acid-induced IBD in rats.

Male Sprague Dawley rats (10 weeks old, 200-250 gr, Harlan, Israel) are lightly anaesthetized by i.p. injection of a ketamine:rompun combination (100:10 mg/kg

30   respectively). A polyethylene catheter (outer diameter 1.7 mm) is inserted through the rectum 5 cm into the colon. And 2 ml of 5% acetic acid are then slowly administered into the colon. Fifteen seconds later the colon is washed with 3 ml saline and 15 seconds later with additional

3 ml of saline. Immediately after, each group of animals is treated with either one of the appropriate treatments. All treatments are administered once daily for 7 days. Animals are clinically followed for 1 week. During this period, the following parameters were daily monitored and recorded: body weight, presence of blood in the stool and stool consistency.

5   These findings are scored according to table 1.

Table 1: Criteria for Scoring Disease Activity Index (DAI[#]) of IBD

(Murthy S.N. et al., Dig. Dis. Sci. 38: 1722-34, 1993).

Score	Weight Loss (%)	Stool Consistency *	Occult Blood or Gross Bleeding
0	None	Normal	Negative
1	1-5	Loose Stool	Negative
2	5-10	Loose Stool	Hemoccult Positive
3	10-15	Diarrhea	Hemoccult Positive
4	>15	Diarrhea	Gross Bleeding

[#]   DAI– (combined score of weight loss, stool consistency, and bleeding)/3.

10   *   Normal stool - well formed pellets; loose stools - pasty stool that does not stick to the anus; and diarrhea - liquid stools that sticks to the anus.

Seven days post disease induction animals are sacrificed with pentobarbital 100 mg/kg i.p. The whole colon is excised, slit longitudinally and examined under a magnifying glass, and any visible damage is recorded and scored according to table 2.

15   **Table 2:** Gross Pathology Scoring Method for Evaluating the Severity of IBD

(Wong et al., J. Pharm. Exp. Ther. 274: 475-80, 1995).

Score	Pathology
0	No damage
1	Localized hyperemia and/or edema
2	Two or sites of hyperemia and/or edema
3	Localized erosion
4	Localized ulcer
5	More then 1 site of erosion/or ulcer, or 1 erosion site or ulcer extending > 2 cm along the length of the colon

The clinical outcome is analyzed using analysis of variance (ANOVA) followed by Duncan's post-hoc test. A non-parametric test (Wilcoxon Rank Sum Test) is used for evaluating the gross pathology findings.
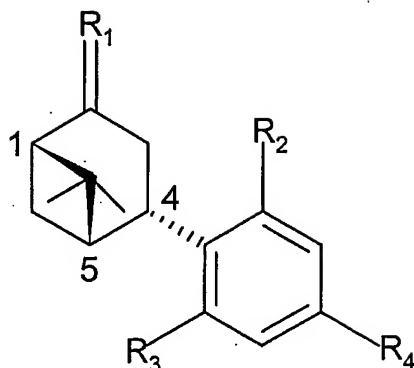
5

Although the present invention has been described with respect to various specific embodiments presented thereof for the sake of illustration only, such specifically disclosed embodiments should not be considered limiting. Many other such embodiments will occur to those skilled in the art based upon applicants' disclosure herein, and applicants propose to be bound only by the spirit and scope of their invention as defined in the appended claims.

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CLAIMS

1. . A compound of the general formula (I):

Formula I

having a specific stereochemistry wherein C-5 is S, the protons at C-1 and C-5 are cis in relation to one another and the protons at C-4 and C-5 are trans; wherein:

R_1 is selected from the group consisting of

(a) O,

(b) S,

(c) $C(R')_2$ wherein each R' is independently selected from the group consisting of hydrogen, cyano, -OR", -N(R")$_2$, a saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl, C_1-C_6 alkyl-OR" or C_1-C_6 alkyl-N(R")$_2$ wherein at each occurrence R" is independently selected from the group consisting of hydrogen, C(O)R''', C(O)N(R''')$_2$, C(S)R''', saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl, C_1-C_6 alkyl-OR''', and C_1-C_6 alkyl-N(R''')$_2$, wherein at each occurrence R''' is independently selected from the group consisting of hydrogen or saturated or unsaturated, linear, branched or cyclic C_1-C_{12} alkyl, and

(d) NR^a wherein R^a is selected from the group consisting of a hydrogen, R" and -OR" wherein R" is as previously defined;

R_2 and R_3 are each independently selected from the group consisting of

(a) halogen,

(b) -R", -OR", -N(R")$_2$, -SR", -S(O)(O)NR", wherein at each occurrence R" is as previously defined, and

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(c) $-S(O)R^b$, $-S(O)(O)R^b$, $-S(O)(O)OR^b$ wherein R^b is selected from the group consisting of hydrogen, saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl, C_1-C_6 alkyl-OR", and C_1-C_6 alkyl-N(R")$_2$, wherein R" is as previously defined; and

5   R_4 is selected from the group consisting of

(a) R wherein R is selected from the group consisting of hydrogen, OR''', OC(O)R''', C(O)OR''', C(O)R''', OC(O)OR''', N(R''')$_2$, NC(O)R''', NC(O)OR''', C(O)N(R''')$_2$, NC(O)N(R''')$_2$, SR''', and C(S)R''', wherein at each occurrence R''' is as previously defined,

10   (b) a saturated or unsaturated, linear, branched or cyclic C_1-C_{12} alkyl-R wherein R is as previously defined,

(c) an aromatic ring which can be further substituted at any position by a halogen, CN, NO$_2$ and R wherein R is as previously defined, and

(d) a saturated or unsaturated, linear, branched or cyclic C_1-C_{12} alkyl optionally

15   terminated by an aromatic ring which can be further substituted as defined in (c).
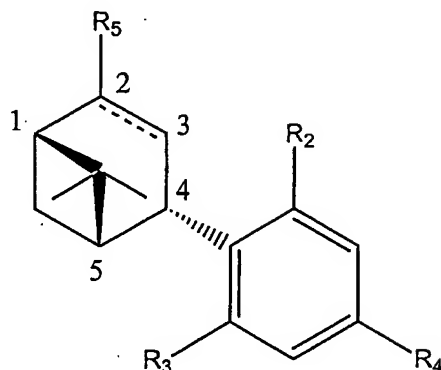
2.   The compound of claim 1 wherein R_1 is O, R_2 and R_3 are OH and R_4 is 1,1-dimethylheptyl.

3.   The compound of claim 1 wherein R_1 is O, R_2 and R_3 are OCH$_3$ and R_4 is 1,1-dimethylheptyl.

20   4.   The compound of claim 1 wherein R_1 is NOH, R_2 and R_3 are OH and R_4 is 1,1-dimethylheptyl.

5.   The compound of claim 1 wherein R_1 is O, R_2 and R_3 are OH and R_4 is 1,1-dimethylhept-6-ynyl.

6.   The compound of claim 1 wherein R_1 is O, R_2 and R_3 are OH and R_4 is 1,1- dimethyl-

25   3-phenylpropyl.

7.   The compound of claim 1 wherein R_1 is O, R_2 and R_3 are OH and R_4 is 1,1,3-trimethylbutyl.

8.   The compound of claim 1 wherein R_1 is O, R_2 and R_3 are OH and R_4 is 1,1- dimethyl-p-chlorobenzyl.

9.   The compound of claim 1 wherein R_1 is O, R_2 and R_3 are OH and R_4 is 1,1-dimethypentyl.

10.   A compound of the general formula (II):

Formula II

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having a specific stereochemistry wherein C-5 is S, the protons at C-1 and C-5 are cis in relation to one another, the protons at C-4 and C-5 are trans, and C-2------C-3 is an optional double bond; wherein:

R_5 is selected from the group consisting of

10   (a) halogen or hydrogen,

            (b) –OR", –N(R")$_2$, –SR", –S(O)(O)NR",   wherein at each occurrence R" is independently selected from the group consisting of hydrogen, C(O)R''', C(O)N(R''')$_2$, C(S)R''', saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl, C_1-C_6 alkyl-OR''', and C_1-C_6 alkyl-N(R''')$_2$, wherein at each occurrence R''' is independently

15   selected from the group consisting of hydrogen or saturated or unsaturated, linear, branched or cyclic C_1-C_{12} alkyl,

            (c) a saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl–SR" or C_1-C_6 alkyl–S(O)(O)NR", wherein R" is as previously defined,

            (d) –S(O)R^b , –S(O)(O)R^b, –S(O)(O)OR^b wherein R^b is selected from the group

20   consisting of hydrogen, saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl, C_1-C_6 alkyl-OR", and C_1-C_6 alkyl-N(R")$_2$, wherein at each occurrence R" is as previously defined,

            (e) a saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl–S(O)R^b , C_1-C_6 alkyl–S(O)(O)R^b, C_1-C_6 alkyl–S(O)(O)OR^b wherein R^b is as previously defined, and

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(f) $-R^c$ wherein R^c is selected from the group consisting of saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl, C_1-C_6 alkyl-OR", C_1-C_6 alkyl-N(R")$_2$, C_1-C_6 alkyl-C(O)OR", and C_1-C_6 alkyl-C(O)N(R")$_2$ wherein at each occurrence R" is as previously defined;

5   R_2 and R_3 are each independently selected from the group consisting of

(a) halogen,

(b) $-R$", $-OR$", $-N(R")_2$, $-SR$", $-S(O)(O)NR$", wherein at each occurrence R" is as previously defined, and

(c) $-S(O)R^b$, $-S(O)(O)R^b$, $-S(O)(O)OR^b$ wherein R^b is as previously defined; and

10   R_4 is selected from the group consisting of

(a) R wherein R is selected from the group consisting of hydrogen, OR''', OC(O)R''', C(O)OR''', C(O)R''', OC(O)OR''', N(R''')$_2$, NC(O)R''', NC(O)OR''', C(O)N(R''')$_2$, NC(O)N(R''')$_2$, SR''', and C(S)R''', wherein at each occurrence R''' is as previously defined,

15   (b) a saturated or unsaturated, linear, branched or cyclic C_1-C_{12} alkyl-R wherein R is as previously defined,

(c) an aromatic ring which can be further substituted at any position by a halogen, CN, NO_2 and R wherein R is as previously defined, and

(d) a saturated or unsaturated, linear, branched or cyclic C_1-C_{12} alkyl optionally

20   terminated by an aromatic ring which can be further substituted as defined in (c);

with the proviso that when R_5 is R^c, then R_4 cannot be a straight or branched saturated C_1-C_{12} alkyl chain optionally preceded or interrupted by an oxygen atom and optionally substituted at the terminal carbon by a hydroxyl or a phenyl group.

11.   The compound of claim 10 wherein R_5 is OH, R_2 and R_3 are OH, R_4 is 1,1-

25   dimethylheptyl and there is a single bond between C-2 and C-3.

12.   The compound of claim 10 wherein R_5 is CH_3, R_2 and R_3 are OH, R_4 is 1,1-dimethylheptynyl and there is a double bond between C-2 and C-3.

13.   A pharmaceutical composition comprising as an active ingredient a compound of the general formula (I):

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Formula I
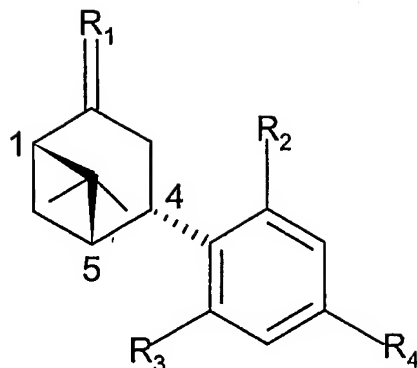
having a specific stereochemistry such that C-5 is S, the protons at C-1 and C-5 are cis in relation to one another and the protons at C-4 and C-5 are trans; wherein:

5   R_1 is selected from the group consisting of

(a) O,

(b) S,

(c) $C(R')_2$ wherein each R' is independently selected from the group consisting of hydrogen, cyano, -OR", -N(R")$_2$, a saturated or unsaturated, linear, branched or cyclic

10   C_1-C_6 alkyl, C_1-C_6 alkyl-OR" or C_1-C_6 alkyl-N(R")$_2$ wherein at each occurrence R" is independently selected from the group consisting of hydrogen, C(O)R''', C(O)N(R''')$_2$, C(S)R''', saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl, C_1-C_6 alkyl-OR''', and C_1-C_6 alkyl-N(R''')$_2$, wherein at each occurrence R''' is independently selected from the group consisting of hydrogen or saturated or unsaturated, linear,

15   branched or cyclic C_1-C_{12} alkyl, and

(d) NR^a wherein R^a is selected from the group consisting of a hydrogen, R" and -OR" wherein R" is as previously defined;

R_2 and R_3 are each independently selected from the group consisting of

(a) halogen,

20   (b) –R", –OR", –N(R")$_2$, –SR", –S(O)(O)NR", wherein at each occurrence R" is as previously defined, and

(c) –S(O)R^b , –S(O)(O)R^b, –S(O)(O)OR^b wherein R^b is selected from the group consisting of hydrogen, saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl, C_1-C_6 alkyl-OR", and C_1-C_6 alkyl-N(R")$_2$, wherein R" is as previously defined;

25   and

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R_4 is selected from the group consisting of

     (a) R wherein R is selected from the group consisting of hydrogen, OR''', OC(O)R''', C(O)OR''', C(O)R''', OC(O)OR''', N(R''')$_2$, NC(O)R''', NC(O)OR''', C(O)N(R''')$_2$, NC(O)N(R''')$_2$, SR''', and C(S)R''', wherein at each occurrence R''' is as previously defined,

     (b) a saturated or unsaturated, linear, branched or cyclic C_1-C_{12} alkyl-R wherein R is as previously defined,

     (c) an aromatic ring which can be further substituted at any position by a halogen, CN, NO_2 and R wherein R is as previously defined, and

     (d) a saturated or unsaturated, linear, branched or cyclic C_1-C_{12} alkyl optionally terminated by an aromatic ring which can be further substituted as defined in (c);

and further comprising a pharmaceutically acceptable diluent or carrier.

14.   The pharmaceutical composition of claim 13 wherein R_1 is O, R_2 and R_3 are OH and R_4 is 1,1-dimethylheptyl.

15.   The pharmaceutical composition of claim 13 wherein R_1 is O, R_2 and R_3 are OCH_3 and R_4 is 1,1-dimethylheptyl.

16.   The pharmaceutical composition of claim 13 wherein R_1 is NOH, R_2 and R_3 are OH and R_4 is 1,1-dimethylheptyl.

17.   The pharmaceutical composition of claim 13 wherein R_1 is O, R_2 and R_3 are OH and R_4 is 1,1-dimethylhept-6-ynyl.

18.   The pharmaceutical composition of claim 13 wherein R_1 is O, R_2 and R_3 are OH and R_4 is 1,1-dimethyl-3-phenylpropyl.

19.   The pharmaceutical composition of claim 13 wherein R_1 is O, R_2 and R_3 are OH and R_4 is 1,1,3-trimethylbutyl.

20.   The pharmaceutical composition of claim 13 wherein R_1 is O, R_2 and R_3 are OH and R_4 is 1,1-dimethyl-p-chlorobenzyl.

21.   The pharmaceutical composition of claim 13 wherein R_1 is O, R_2 and R_3 are OH and R_4 is 1,1-dimethylpentyl.

22.   A pharmaceutical composition comprising as an active ingredient a compound of the general formula (II):

Formula II
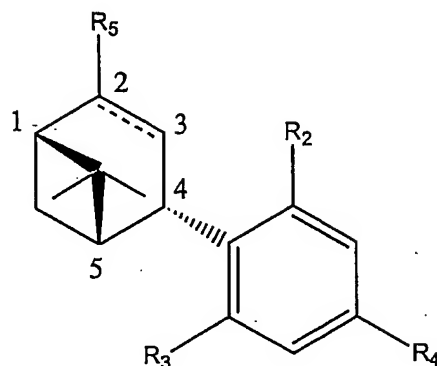
5   having a specific stereochemistry wherein C-5 is S, the protons at C-1 and C-5 are cis in relation to one another, the protons at C-4 and C-5 are trans, and C-2------C-3 is an optional double bond; wherein:

R_5 is selected from the group consisting of

(a) halogen or hydrogen,

10   (b) $-OR"$, $-N(R")_2$, $-SR"$, $-S(O)(O)NR"$, wherein at each occurrence $R"$ is independently selected from the group consisting of hydrogen, $C(O)R"'$, $C(O)N(R"')_2$, $C(S)R"'$, saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl, C_1-C_6 alkyl-$OR"'$, and C_1-C_6 alkyl-$N(R"')_2$, wherein at each occurrence $R"'$ is independently selected from the group consisting of hydrogen or saturated or unsaturated, linear,

15   branched or cyclic C_1-C_{12} alkyl,

(c) a saturated or unsaturated, linear, branched or. cyclic C_1-C_6 alkyl-$SR"$ or C_1-C_6 alkyl-$S(O)(O)NR"$, wherein $R"$ is as previously defined,

(d) $-S(O)R^b$, $-S(O)(O)R^b$, $-S(O)(O)OR^b$ wherein R^b is selected from the group consisting of hydrogen, saturated or unsaturated, linear, branched or cyclic C_1-C_6

20   alkyl, C_1-C_6 alkyl-$OR"$, and C_1-C_6 alkyl-$N(R")_2$, wherein at each occurrence $R"$ is as previously defined,

(e) a saturated or unsaturated, linear, branched or cyclic C_1-C_6 alkyl-$S(O)R^b$, C_1-C_6 alkyl-$S(O)(O)R^b$, C_1-C_6 alkyl-$S(O)(O)OR^b$ wherein R^b is as previously defined, and

(f) $-R^c$ wherein R^c is selected from the group consisting of saturated or unsaturated,

25   linear, branched or cyclic C_1-C_6 alkyl, C_1-C_6 alkyl-$OR"$, C_1-C_6 alkyl-$N(R")_2$, C_1-C_6

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alkyl-C(O)OR", and C_1-C_6 alkyl-C(O)N(R")$_2$ wherein at each occurrence R" is as previously defined;

R_2 and R_3 are each independently selected from the group consisting of

(a) halogen,

(b) –R", –OR", –N(R")$_2$, –SR", –S(O)(O)NR", wherein at each occurrence R" is as previously defined, and

(c) –S(O)R^b, –S(O)(O)R^b, –S(O)(O)OR^b wherein R^b is as previously defined; and

R_4 is selected from the group consisting of

(a) R wherein R is selected from the group consisting of hydrogen, OR''', OC(O)R''', C(O)OR''', C(O)R''', OC(O)OR''', N(R''')$_2$, NC(O)R''', NC(O)OR''', C(O)N(R''')$_2$, NC(O)N(R''')$_2$, SR''', and C(S)R''', wherein at each occurrence R''' is as previously defined,

(b) a saturated or unsaturated, linear, branched or cyclic C_1-C_{12} alkyl-R wherein R is as previously defined,

(c) an aromatic ring which can be further substituted at any position by a halogen, CN, NO$_2$ and R wherein R is as previously defined, and

(d) a saturated or unsaturated, linear, branched or cyclic C_1-C_{12} alkyl optionally terminated by an aromatic ring which can be further substituted as defined in (c);

with the proviso that when R_5 is R^c, then R_4 cannot be a straight or branched saturated C_1-C_{12} alkyl chain optionally preceded or interrupted by an oxygen atom and optionally substituted at the terminal carbon by a hydroxyl or a phenyl group;

and further comprising a pharmaceutically acceptable diluent or carrier.

23.   The pharmaceutical composition of claim 22 wherein R_5 is OH, R_2 and R_3 are OH, R_4 is 1,1-dimethylheptyl and there is a single bond between C-2 and C-3.

24.   The pharmaceutical composition of claim 22 wherein R_5 is CH$_3$, R_2 and R_3 are OH, R_4 is 1,1-dimethylheptynyl and there is a double bond between C-2 and C-3.

25.   The pharmaceutical composition according to any one of claims 13 to 24 wherein the diluent comprises an aqueous cosolvent solution comprising a pharmaceutically acceptable cosolvent, a micellar solution or emulsion prepared with natural or synthetic ionic or non-ionic surfactants, or a combination of such cosolvent and micellar or emulsion solutions.

26.   The pharmaceutical composition according to any one of claims 13 to 24 wherein the carrier comprises a solution of ethanol, a surfactant and water.

27.   The pharmaceutical composition according to any one of claims 13 to 24 wherein the carrier is an emulsion comprising triglycerides, lecithin, glycerol, an emulsifier, and water.

5   28.   The pharmaceutical composition according to any one of claims 13 to 24 in unit dosage form.

29.   The pharmaceutical composition according to claim 28 suitable for oral administration.

30.   The pharmaceutical composition according to claim 28 suitable for parenteral
10   administration.

31.   A method for preventing, alleviating or treating a disease or disorder amenable to CB2 receptor modulation, by administering to an individual in need thereof of a therapeutically effective amount of a pharmaceutical composition comprising as an active ingredient a compound according to any one of claims 1 to 12.

15   32.   A method for preventing, alleviating or treating autoimmune disease and inflammation, rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, myasthenia gravis, diabetes mellitus type I, psoriasis, inflammatory bowel disease, tissue rejection in organ transplants, malabsorption syndromes, celiac disease, pulmonary disease, asthma and Sjögren's syndrome, by administering to an individual in need thereof of a
20   therapeutically effective amount of a pharmaceutical composition comprising as an active ingredient a compound according to any one of claims 1 to 12.

33.   A method for preventing, alleviating or treating neurological disorders, stroke, migraine, cluster headache, neurodegenerative diseases, Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, Huntington's chorea, prion-associated diseases,
25   poisoning of the central nervous system, and muscle spasm and tremor, by administering to an individual in need thereof of a therapeutically effective amount of a pharmaceutical composition comprising as an active ingredient a compound according to any one of claims 1 to 12.

34.   A method for preventing, alleviating or treating pain including peripheral, neuropathic
30   and referred pain, by administering to an individual in need thereof of a therapeutically

effective amount of a pharmaceutical composition comprising as an active ingredient a compound according to any one of claims 1 to 12.

35.   A method for preventing, alleviating or treating cardiovascular disorders, arrhythmia, hypertension and myocardial ischemic damage, by administering to an individual in need
5   thereof of a therapeutically effective amount of a pharmaceutical composition comprising as an active ingredient a compound according to any one of claims 1 to 12.

36.   A method for preventing, alleviating or treating cancer, by administering to an individual in need thereof of a therapeutically effective amount of a pharmaceutical composition comprising as an active ingredient of a compound according to any one of claims
10   1 to 12.

37.   A method for preventing, alleviating or treating neuropathic pain, by administering to an individual in need thereof of a therapeutically effective amount of a pharmaceutical composition comprising as an active ingredient of a compound of general formula II wherein R_5 is CH_2OH, R_2 and R_3 are OCH_3, R_4 is 1,1-dimethylheptyl and there is a double bond
15   between C-2 and C-3.

38.   A method for preventing, alleviating or treating Parkinson's disease, by administering to an individual in need thereof of a therapeutically effective amount of a pharmaceutical composition comprising as an active ingredient of a compound of general formula II wherein R_5 is CH_2OH, R_2 and R_3 are OCH_3, R_4 is 1,1-dimethylheptyl and there is a double bond
20   between C-2 and C-3.

39.   The method of any one of claims 31-38 wherein the composition is administered orally, parenterally, intravenously, intramuscularly, intralesionally, subcutaneously, transdermally, intrathecally, rectally and intranasally.

40.   Use for the preparation of a medicament for preventing, alleviating or treating a
25   disease or disorder amenable to CB2 receptor modulation, of a compound according to any one of claims 1 to 12.

41.   Use for the preparation of a immunomodulatory and anti-inflammatory medicament for preventing, alleviating or treating autoimmune disease and inflammation, rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, myasthenia gravis, diabetes
30   mellitus type I, psoriasis, inflammatory bowel disease, tissue rejection in organ transplants,

malabsorption syndromes, celiac disease, pulmonary disease, asthma and Sjögren's syndrome, of a compound according to any one of claims 1 to 12.

42.   Use for the preparation of a neuroprotective medicament for preventing, alleviating or treating neurological disorders, stroke, migraine, cluster headache, neurodegenerative diseases, Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, Huntington's chorea, prion-associated diseases, poisoning of the central nervous system, and muscle spasm and tremor, of a compound according to any one of claims 1 to 12.

43.   Use for the preparation of an analgesic medicament for preventing, alleviating or treating pain including peripheral, neuropathic and referred pain, of a compound according to any one of claims 1 to.12.

44.   Use for the preparation of a medicament for preventing, alleviating or treating cardiovascular disorders, arrhythmia, hypertension and myocardial ischemic damage, of a compound according to any one of claims 1 to 12.

45.   Use for the preparation of a medicament for preventing, alleviating or treating cancer, of a compound according to any one of claims 1 to 12.

46.   Use for the preparation of a medicament for preventing, alleviating or treating neuropathic pain of a compound of general formula II wherein R_5 is CH_2OH, R_2 and R_3 are OCH_3, R_4 is 1,1-dimethylheptyl and there is a double bond between C-2 and C-3. `
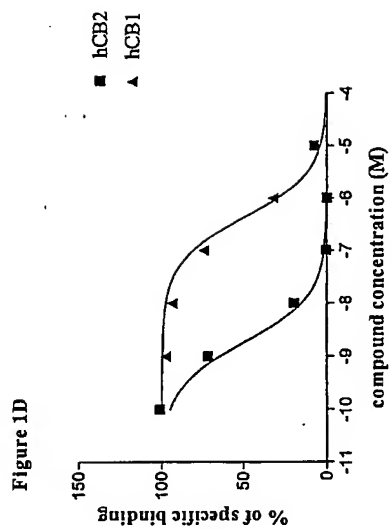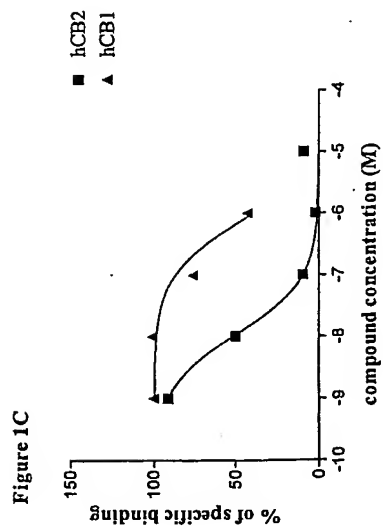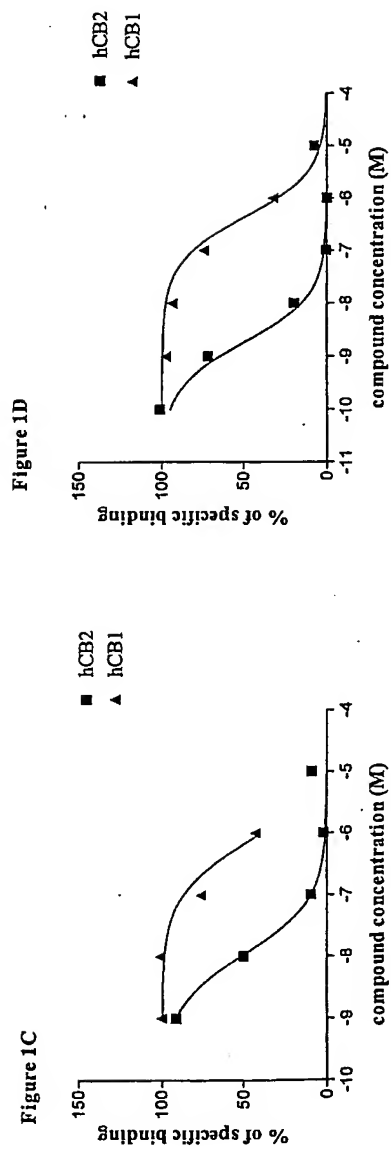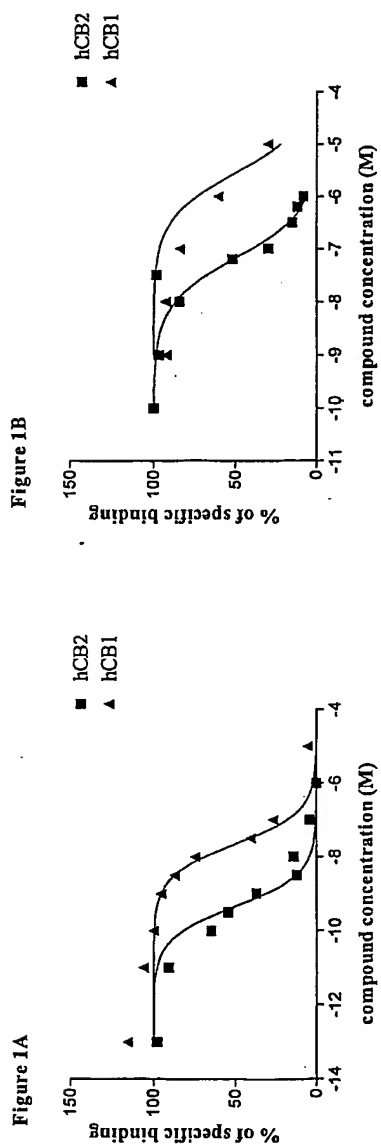
47.   · Use for the preparation of a medicament for preventing, alleviating or treating Parkinson's disease, of a compound of general formula II wherein R_5 is CH_2OH, R_2 and R_3 are OCH_3, R_4 is 1,1-dimethylheptyl and there is a double bond between C-2 and C-3.

Figure 1A

Figure 1B

Figure 1C
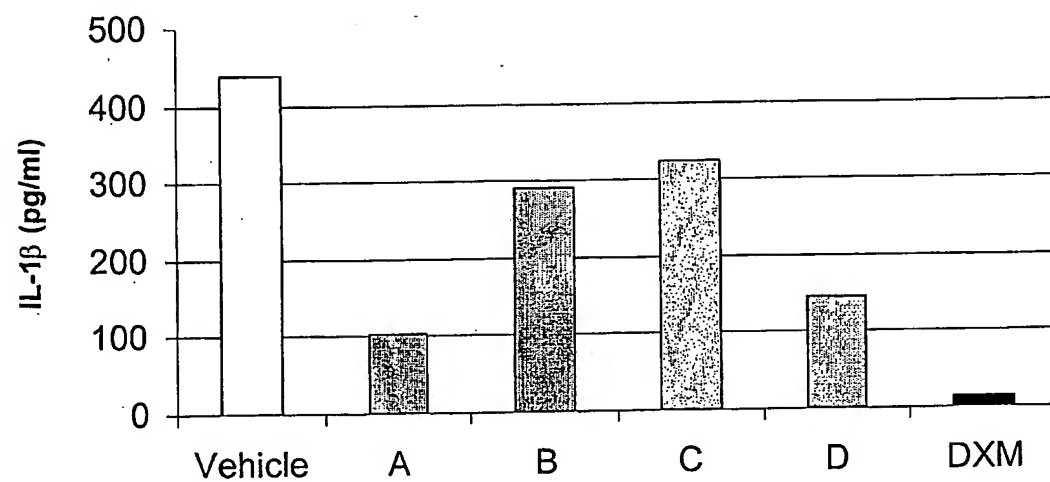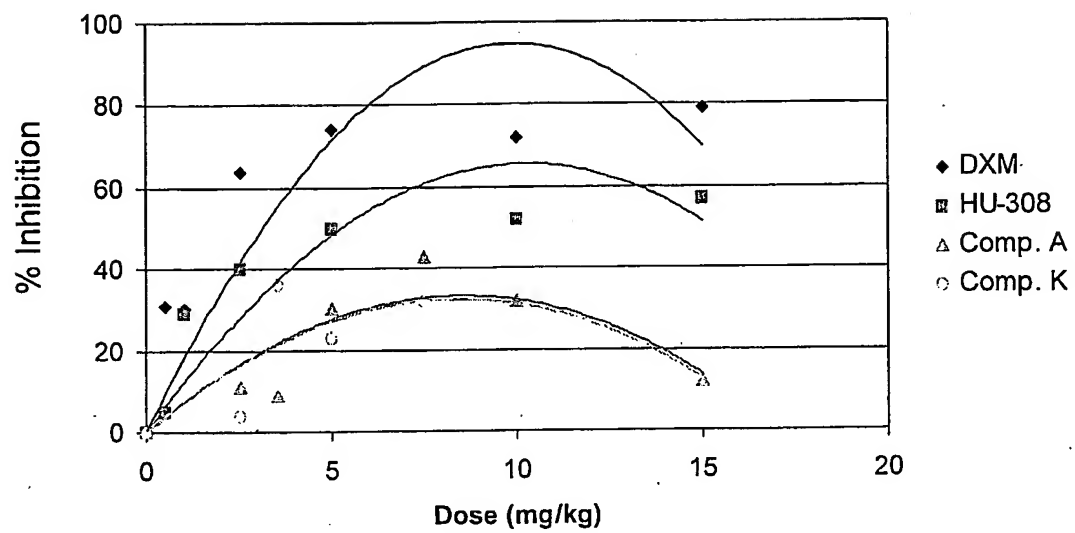
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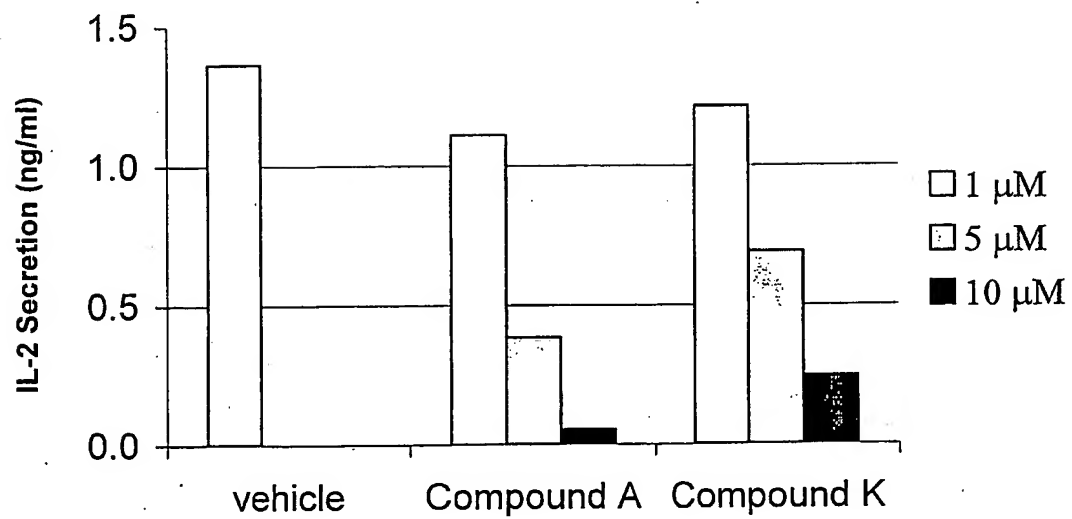
Figure 2

Figure 3

Figure 4
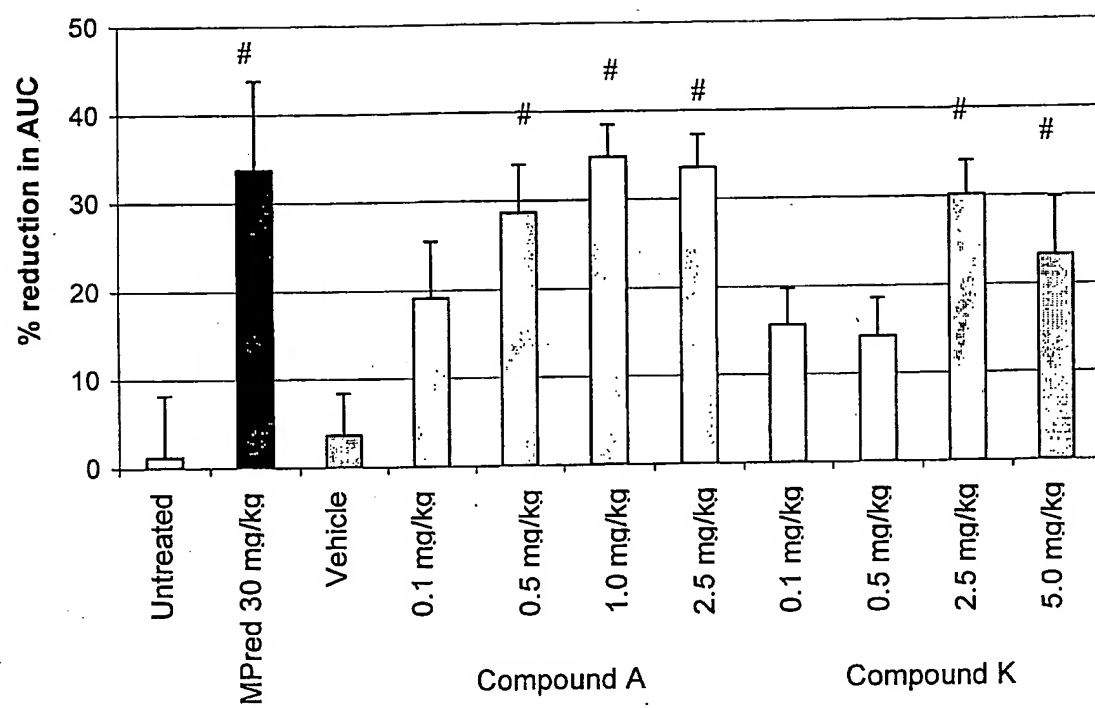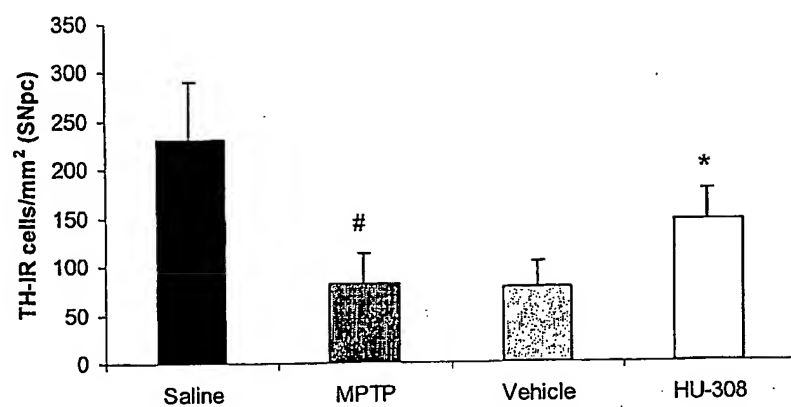
Figure 5

Figure 6

Figure 7
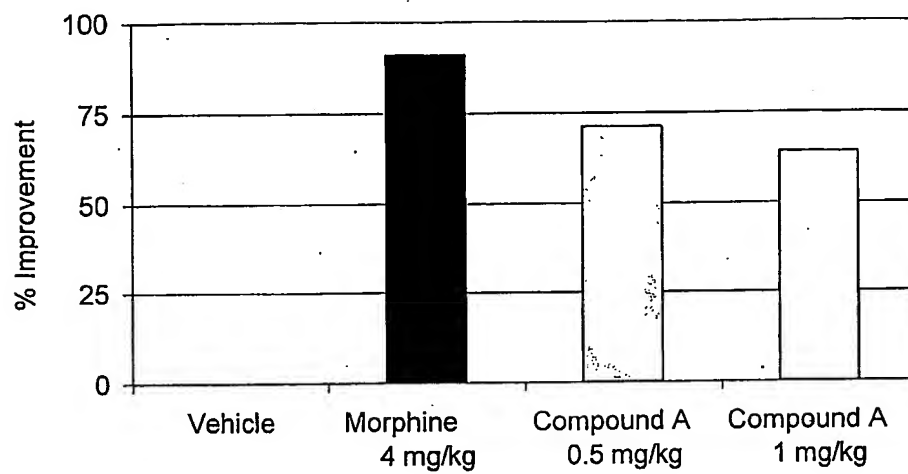
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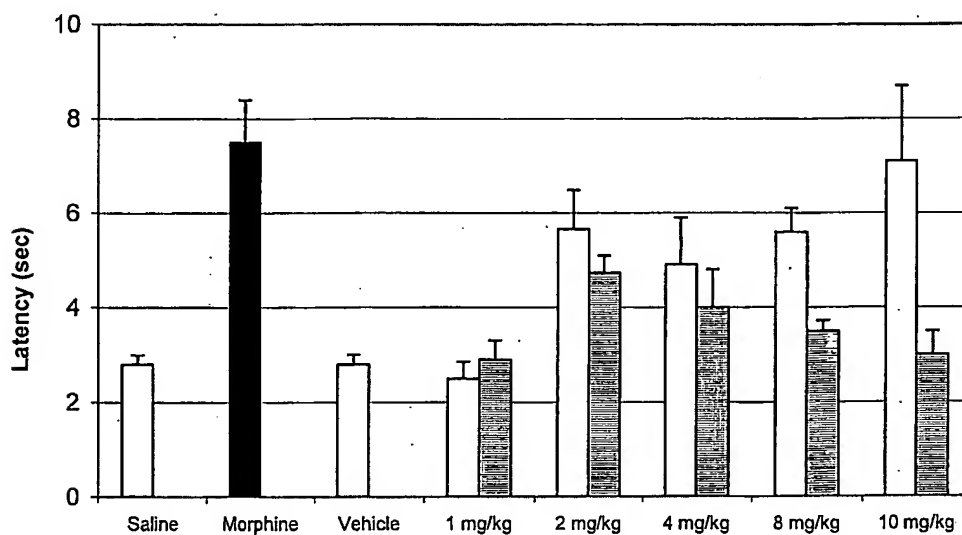
Figure 8B
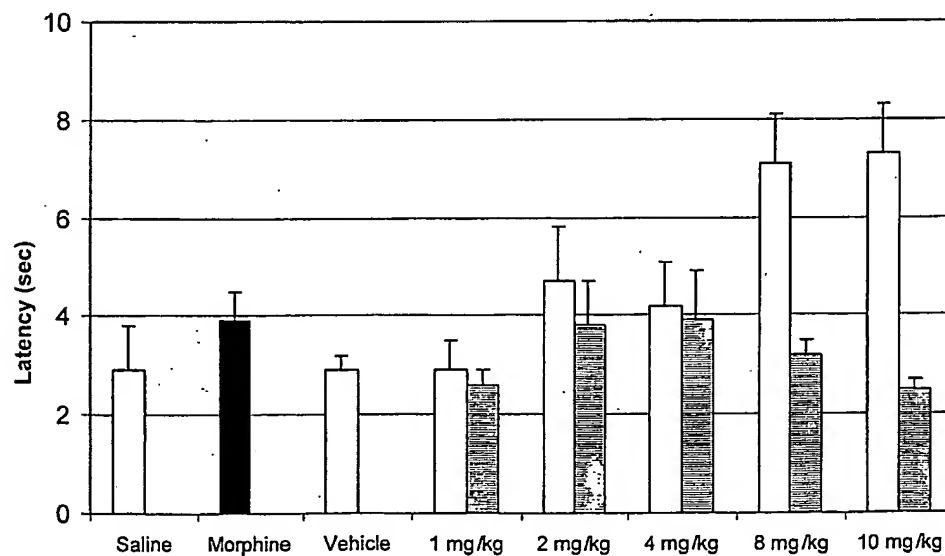
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C07C 39/17, 49/105, 69/01, 315/00; A61K 31/12, 31/35
US CL :568/74, 376, 734; 560/141, 255; 514/454, 511, 680
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 568/74, 376, 734; 560/141, 255; 514/454, 511, 680

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data-base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,282,248 A (MECHOULAM et al.) 04 August 1981(04.08.1981) see entire document.	10-12 and 22-47
X	Database Caplus, Accession Number 1998, Drake et al. Classical/Nonclassical Hybrid Cannabinoids; Journal of Medicinal Chemistry. 1998, Vol. 41, No. 19, pages 3596-3608, abstract.	1-9, 13-21 and 25-47

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

* Special categories of cited documents:

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 AUGUST 2002	16 DEC 2002

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer JOHANN RICHTER
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